ANAIS

X Simpósio do Programa de Pós-graduação em Biologia molecular

23 e 24 de Novembro, 2021 | ON-LINE

IB UnB

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

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

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Sumário / Summary

Apresentação

O X Simpósio do Programa de Pós-Graduação em Ciências Biológicas (Biologia Molecular) (PPGBioMol) da Universidade de Brasília (UnB) aconteceu nos dias 23 e 24 de novembro de 2021. Devido à COVID-19, este evento foi on-line, com a participação de convidados e alunos, pesquisadores e professores da UnB. O simpósio foi aberto para estudantes e profissionais com produção acadêmica ou que tinham interesse em temas moleculares das ciências da vida.

Tendo como base que o PPGBioMol compreende inúmeras linhas de pesquisas, os resumos dos inscritos foram classificados em quatro grandes áreas: (01) Biologia celular, desenvolvimento e câncer; (02) Bioquímica, biofísica e biologia estrutural; (03) Genética, genômica e evolução; (04) Biologia celular e molecular de microrganismos. Foram escolhidos 3 trabalhos de cada área para apresentação oral síncrona no dia 24 de novembro.

Presentation

The X Symposium of the Graduate Program in Biological Sciences (Molecular Biology) (PPGBioMol) of the University of Brasilia occurred on November 23rd and 24th, 2021. Due to COVID-19 pandemy, this event was on-line, with the participation of guests and UnB students, researchers, and professors. The symposium was opened to students and professionals with academic production or who were interested in molecular themes within the life sciences.

As PPGBioMol develop work in numerous research fields, the abstracts were categorized in four major areas: (01) Cellular biology, development, and cancer; (02) Biochemistry, biophysics, and structural biology; (03) Genetics, genomics, and Evolution; (04) Cellular and molecular biology of microorganisms. Three abstracts from each area were selected for synchronous oral presentation on November 24th.

Comissão organizadora / Organizing committee

Professores do Departamento de Biologia Celular

Dra. Andréa Queiroz Maranhão (Coordenadora PPGBioMol)

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Genética, genômica e evolução

Dr. Leonardo Assis da Silva

Biologia celular e molecular de microrganismos

Dr. Athos Silva de Oliveira

Ma. Jacyelle Medeiros

Dra. Paula Beatriz Santiago

Programação / Program

23 de Novembro / Terça-feria	
9:30 – 10:00 h	**Mesa de Abertura** ✣
10:00 – 10:50 h	**O processo regulatório para autorização de vacinas no Brasil** ✣ Ma. Maria Fernanda Reis e Silva Thees Gerente de Avaliação de Produtos Biológicos - ANVISA - Agência Nacional de Vigilância sanitária
11:00 – 11:50 h	**Startup em biotecnologia: desafios e perspectivas** ✣ Profa Dra. Márcia Renata Mortari Departamento de Ciências Fisiológicas - UnB- Universidade de Brasíllia
14:00 – 17:00 h	**Resumos gravados disponíveis para apreciação do público** ✺
24 de Novembro / Quarta-feria	
9:00 – 9:45 h	**Apresentações síncronas Biologia Celular, desenvolvimento e câncer (A1)** ✣ **Evaluation of immune system activation through methylene blue associated with nanostructures for breast cancer in vitro** Ana Luísa de Gouvêa da Silva **The role of omega-3 in white and brown adipose tissue modulation: differential functions involved in the progression of metastatic melanoma** Débora Santos da Silva **Cytotoxicity assay of lipid nanoparticles associated with docetaxel in gastric adenocarcinoma** Laís Vaz da Costa
9:45 – 10:15 h	**Apresentações síncronas Bioquímica, biofísica e biologia estrutural (A2)** ✣ **Structural and electrophysiological evaluation of Nav channel modulators isolated from *Tityus stigmurus* venom** Daniel Oliveira da Mata **Estudos estruturais e funcionais das enzimas esterol C24-metiltransferases dos fungos patogênicos humanos *Candida auris* e *Aspergillus fumigatus* visando o desenvolvimento de potenciais fármacos antifúngicos** Gideane Mendes de Oliveira **Kinetic and structural study of the interaction between ferulic acid and HXYN2 endoxylanase from *Humicola grisea* var. *thermoidea*** Izadora Cristina Moreira de Oliveira
10:45 – 11:15 h	**Apresentações síncronas Genética, genômica e evolução (A3)** ✣ **Scalable and versatile pipelines for comprehensive bacterial genomics analyses** Felipe Marques de Almeida **Contrasting transcriptomic profiles in resistant and susceptible cupuassu (*Theobroma grandiflorum*) challenged with *Moniliophthora perniciosa*, the causal agent of witches' broom disease** Loeni Lüdke Falcão **Ship: Annotation-based program for identifying Genomic Safe Harbours in eukaryotic model organisms** Matheus de Castro Leitão
11:30 – 12:15 h	**Apresentações síncronas Biologia celular e molecular de microorganismos (A4)** ✣ **Análise comparativa entre componentes extracelulares secretados por isolados do Fonsecaea sp a partir de diferentes condições de cultivo na modulação da resposta imune inata do hospedeiro** Lucas de Oliveira Las-Casas **Construction of infectious clone of cucurbit aphid-borne yellows virus brazilian melon isolate** Thiago Marques Costa **RNAi-Mediated Parasitism Gene Silencing: A Novel Source of Resistance for Crop Protection against the *Meloidogyne incognita*** Valdeir Junio Vaz Moreira
14:00 – 14:50 h	**A tragédia dos trópicos: calor, pobreza e doenças** ✣ Prof. Dr. Carlos H. N. Costa Instituto de Doenças do Sertão – UFPI
15:00 – 15:30 h	**Encerramento** ✣

✣ Apresentações disponíveis no canal do IB Virtual - UnB
✺ Playlist “Resumos” disponível no canal PPG Biomol

Palestras / Invited talks

P1 - O processo regulatório para autorização de vacinas no Brasil
Ma. Maria Fernanda Reis e Silva Thees
Gerente de Avaliação de Produtos Biológicos - ANVISA - Agência Nacional de Vigilância sanitária
Brasília – DF

P2 - Startup em biotecnologia: desafios e perspectivas
Profa. Dra. Márcia Renata Mortari
Departamento de Ciências Fisiológicas - UnB- Universidade de Brasíllia
Brasília – DF

P3 - A tragédia dos trópicos: calor, pobreza e doenças
Prof. Dr. Carlos H. N. Costa
Instituto de Doenças do Sertão – UFPI

Apresentações orais síncronas / Synchronous oral presentations

1. Biologia Celular, desenvolvimento e câncer (A1)

Evaluation of immune system activation through methylene blue associated with nanostructures for breast cancer in vitro
Ana Luísa de Gouvêa da Silva

The role of omega-3 in white and brown adipose tissue modulation: differential functions involved in the progression of metastatic melanoma
Débora Santos da Silva

Cytotoxicity assay of lipid nanoparticles associated with docetaxel in gastric adenocarcinoma
Laís Vaz da Costa

2. Bioquímica, biofísica e biologia estrutural (A2)

Structural and electrophysiological evaluation of Nav channel modulators isolated from *Tityus stigmurus* venom
Daniel Oliveira da Mata

Structural and functional studies of C24-methyltransferase sterol enzymes from human pathogenic fungi *Candida auris* and *Aspergillus fumigatus* aiming at the development of potential antifungal drugs
Gideane Mendes de Oliveira

Kinetic and structural study of the interaction between ferulic acid and HXYN2 endoxylanase from *Humicola grisea* var. *thermoidea*
Izadora Cristina Moreira de Oliveira

3. Genética, genômica e evolução (A3)

Scalable and versatile pipelines for comprehensive bacterial genomics analyses
Felipe Marques de Almeida

Contrasting transcriptomic profiles in resistant and susceptible cupuassu (*Theobroma grandiflorum*) challenged with *Moniliophthora perniciosa*, the causal agent of witches' broom disease
Loeni Lüdke Falcão

Ship: Annotation-based program for identifying Genomic Safe Harbours in eukaryotic model organisms
Matheus de Castro Leitão

4. Biologia celular e molecular de microorganismos (A4)

Comparative analysis between extracellular components secreted by *Fonsecaea* sp isolates from different culture conditions in modulating the host's innate immune response
Lucas de Oliveira Las-Casas

Construction of infectious clone of cucurbit aphid-borne yellows virus brazilian melon isolate
Thiago Marques Costa

RNAi-Mediated Parasitism Gene Silencing: A Novel Source of Resistance for Crop Protection against the *Meloidogyne incognita*
Valdeir Junio Vaz Moreira

O conteúdo destas apresentações encontra-se na seção Resumos.

Resumos / Abstracts

1. Biologia Celular, desenvolvimento e câncer (A1)

A1.R1 - Phylogenetic approach to breast cancer genes

Camylle Monteiro Silva; Marcos Antonio Nobrega de Sousa.
Universidade Federal de Campina Grande (UFCG)

According to the World Health Organization (WHO), in the year 2020 alone, the number of cases of breast cancer in the world was more than two million cases, being the cancer with the highest occurrence in the last 5 years. Of the various genes involved in the development of breast cancer due to genetic and hereditary factors, changes in the BRCA1 and BRCA2 genes receive great attention, due to the increased risk of developing the disease, also influencing this risk, new genetic links are being discovered regularly, in genes such as PALB2, CHEK2 and CDH1. The phylogenetic analysis of genes related to breast cancer is a point of great importance for the prediction and prevention of diseases. The aim of this work was to phylogenetically analyze the evolutionary relationship of genes: BRCA1, BRCA2, PALB2, CHEK2 and CDH1.Using bioinformatics tools, the gene sequences were acquired from the NCBI orthologs database. They were aligned and the trees were generated through an evolutionary methodology applying the bootstrap resampling test, to choose the best tree for the relationships between the species. As a result, it was observed that for BRCA1 and BRCA2, the best model for grouping the studied species was the maximum parsimony, for the genes PALB2 and CHEK2 it was the UPGMA methodology and for CDH1 all 5 models showed a good result. The high rate of evolutionary conservation of these sequences and the importance of using bioinformatics databases can be seen.

A1.R2 - Evaluation of the possible first specific Cav2.3 blocker from scorpion venom and its potential neuroprotective effect in Parkinson's disease

Diogo Vieira Tibery; Adolfo Carlos Barros de Souza; Beatriz Sarmiento Certuche; Elisabeth Ferroni Schwartz.
Neuropharmacology Laboratory, University of Brasília (UNB), Brasília, Distrito Federal

In the venom of the *Opisthacanthus cayaporum* scorpion, 80 fractions were detected by HPLC and 221 components were identified by mass spectrometry with a variation from 229 to 61144 DA. Among these components, Toxin-1 was identified as an inhibitor of Cav2.3 channel currents at a concentration of 300nM, without changing the channel activation and inactivation kinetics. Toxin-1 is a possible specific blocker of this channel, since it was characterized the absence of activity in other voltage-dependent calcium channels (CaV1.2, CaV1.3s), voltage-dependent potassium channels (KV1.3 and KV2.1), calcium-dependent potassium channel (BK) and in ATP-dependent potassium channel (KATP). Voltage-dependent calcium channels (CaV) are membrane proteins responsible for the influx of calcium that allows both the transmission of the electrical signal across the cell membrane and intracellular transduction through the influx of the Ca2+ ion that acts as a second messenger capable of triggering physiological reactions, including muscle contraction, neurotransmitters/hormones secretion and gene regulation. Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies. Dopaminergic neurons in the substantia nigra have pacemaker activity necessary for intracellular Ca2+ oscillations and subsequent dopamine release, such role can induce Ca2+-dependent mitochondrial stress and make neurons more vulnerable. The Cav2.3 channel is one of the components responsible for calcium release in pacemaker activity and animals KO for CaV2.3 present neuroprotection in dopaminergic neurons in the MPTP

model. The aim of this work is the characterization of Toxin-1 activity in ion channels to confirm the specificity for Cav2.3 subtype and evaluation of neuroprotective activity in the animal model for Parkinson's disease.

A1.R3 - The role of alternative splicing in Covid-19

Pedro Henrique Aragão Barros.
Universidade de Brasília (UnB)

Coronavirus disease 2019 (Covid-19) caused by Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) emerged in 2019 and spread around the word. In severe cases, the ability to cause disorders in the immune system has been observed, starting through several described mechanisms and can cause a great release of inflammatory cytokines in a positive feedback system that lead to a cytokine storm. Some of the mechanisms described that lead to this condition is the ability of the virus to interfere in several pathways in the host's cells, leading to a decrease in the release of type I and type III interferons and interfering in a series of secretion pathways that culminate in a delayed antiviral cell state and immune cell dysregulation, which is not yet fully understood. In this project, public available RNA-seq data of peripheral blood mononuclear cells (PBMC) from patients infected with COVID-19 or other viral diseases were obtained. This data was mapped to human reference genome and differential gene expression in order to make comparisons and analyzes of disease-related pathways to understand the immunological mechanisms in COVID-19. Compared to dengue fever, Covid-19 patients differentially express a greater number of genes related to inflammation, while patients with dengue had an increased antiviral response. Several pathways was significantly observed exclusively in covid individuals, with a remarkable decrease in interferon-related pathways, alternative splicing, epigenetic, viral transcription, related to different patterns of immune cells involved in infection. Additionally, nucleotide modification marks were found that could lead to alternative forms of splicing. This RNA editing could be related to an alternative form of the ADAR protein found. The next steps of the project include single-cell RNA-seq analyzes to identify pathways, gene isoforms and base modifications in specific cell types that may be involved in dysregulation of the immune system.

A1.R4 - In silico phylogenetic analysis of BRAF gene for skin cancer

Matheus Medeiros Nunes; Marcos Antonio Nobrega de Sousa; Thaís Lucena de Oliveira.

Skin cancers are divided into two classes, non-melanoma skin cancer and melanomas, and the latter type stands out for having a worse prognosis. In addition, several factors make it difficult to identify and treat cancer, as cancer is the end product of a complex process that develops in multiple stages. Proto-oncogenes are genes responsible for the upregulation of cell proliferation, while tumor suppressor genes are responsible for inhibiting cell multiplication. Skin cancer is caused by the multiplication of abnormal cells, a tumor that affects the skin, and is the most common type of cancer in Brazil, especially in the non-melanoma type, which has lower mortality compared to the melanoma type. The Braf gene participates in biochemical pathways, which act in cell division, differentiation and secretion. The use of genes proved to be an important tool for inferring the relationships between genes. Sequences were acquired from the database for orthologs (NCBI Orthologs), multiple sequence alignment and construction of phylogenetic trees for each of the five evolutionary methods available in the phylogenetic analysis programs. Characteristics conserved among several animal phyla have profound consequences for cancer research, we observed conservation of genes in species that have greater proximity in their evolutionary history, from the five evolutionary methods studied, the best results were selected in each of the methods. Bioinformatics, therefore, has an important role in the development of statistical methods for

analyzing large data sets and in the development of information management methods for the new types of data that are being generated.

A1.R5 - Application of machine learning algorithms in the fight against diseases - case COVID-19

Muller, H. S.; Canavaci, A.M.C.; Martins, V. P.
Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900 Brasília, DF, Brasil. Faculdade de Farmácia, Universidade de Brasília, 70910-900 Brasilia, DF, Brasil.

Over the years, an evolution of interspecies interaction has led both to the development of the eukaryotic immune system in order to eliminate invading microorganisms and the evolution of specific mechanisms for pathogens to enter eukaryotic cells, countering the host's defenses. As a result of this evolution, the emergence of new diseases that may have the potential to cause a pandemic outbreak has left the scientific community on the alert to develop new predictive mechanisms that make it possible to assess an organism-disease interaction in order to combat new diseases and prevent greater harm to society. In order to understand the behavior of this interaction, some scholars develop and apply machine processing models, which would be trained to predict how the response of a certain aspect would be, such as, for example, analysis of microbiome characteristics with a qualified to classify organisms as pathogenic or not, analysis of medical intervention in patients infected by a certain microorganism, analysis of disease prognosis, among others. The aim of this study was to apply machine learning models and select algorithms to create a classifying model of medical intervention to the individual who was hospitalized by COVID-19. The study used public data that were processed, pre-processed and distributed in Python. Among the machine learning algorithms, decision tree, random forest, neural networks, Catboosting and XGboosting were obtained. At the end of the battery of tests, the models that have greater precision and accuracy were chosen to apply to data from large Brazilian cities. Information was generated on how the medical intervention should be carried out before and after vaccination, in addition to verifying the characteristics that had protective and/or aggravating potential. It is concluded that a machine learning application in the health field has become a tool to aid decision making and that it can bring large amounts of information.

A1.R6 - Evaluation of immune system activation through methylene blue associated with nanostructures for breast cancer in vitro

Ana Luísa G. Silva[1]; Leonardo G. Paterno[2]; Cleber L. Filomeno[2]; Sônia N. Báo[1].
[1]Department of Cell Biology, Institute of Biological Sciences, University of Brasília, Brasília/DF – Brazil. [2]Laboratory of Research on Polymers and Nanomaterials, Chemistry Institute, University of Brasília, Brasília/DF – Brazil.

The role of immune system in cancer treatment has promote the development of numberless works in the theme of immunotherapies (Hanahan, et al., 2011). Studies on the cancer biology demonstrate the important cellular characteristics that can be used for its treatment, such as the activation of dendritic cells and CD8+ T lymphocytes (Yarosz, et al., 2018). Nanobiotechnology, the creation of materials at the nanometric scale directed for biology, has been developed and is earning prominence for early diagnosis and targeted drug delivery, in addition to being able to promote an immune response based on the activation of dendritic cells. Previous studies demonstrate efficacy, in vitro, of the use of magnetic nanoparticles associated with methylene blue [MAGCIT-MB] for the treatment of breast and ovarian cancer (Silva, et al., 2021). Thus, the present work included the application of MAGCIT-MB to promote anti-tumor immune responses for the treatment of breast cancer in vitro. As MAGCIT-MB exhibited a hydrodynamic diameter of 60.93 nm with a polydispersion index of 0.199 and zeta potential of -20.9 mV. Transmission electron microscopy (TEM) analysis demonstrate that nanoparticle endocytosis occurs by

phagocytosis in addition to its accumulations in lysosome and/or endosome. The reduction of cell viability was performed with two murine cell lines, one from mammary carcinoma and the other from fibroblast, 4T1 and NIH-3T3, respectively. Both cell lines reduced the viability after treatment with the nanoparticle, however, the results of IC50 analyzes demonstrated greater sensitivity in 4T1 cells. By flow cytometry, the maturation of dendritic cells was analyzed after treatment with the supernatant of treated tumor cells. The results demonstrate that, after stimulation, dendritic cells exhibited changes in cell morphology, acquiring an adherent fusiform shape with cytoplasmic extensions and an increase on the expression of CD80, CD86 and CD11c on their surface.

A1.R7 - Transcriptome analysis of breast cancer cell lines exposed to peptides with potential antitumor activity

Michel Lopes Leite[1]; Nicolau Brito da Cunha[2]; Octávio Luiz Franco[1,3,4]; Nádia Skorupa Parachin[1].

[1]Departamento de Biologia Molecular, Instituto de Ciências Biológicas, Universidade de Brasília, Campus Darcy Ribeiro, Brasília, Distrito Federal, Brasil; [2]Centro de Análises Proteômicas e Bioquímicas, Pós-graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Brasília, Brazil; [3]Universidade de Brasília, Pós-graduação em Patologia Molecular, Campus Darcy Ribeiro, Brasília, Brazil; [4]S-Inova Biotech, Pós-graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil.

The discovery of several tumor suppressor genes and oncogenes in the last two decades has allowed advances in cancer treatment. However, cancer remains a leading cause of death worldwide. Cancer is a term used to bring together in a single group more than one hundred types of diseases that share characteristics such as disordered cell growth, capacity to invade and spread to other organs and tissues. Breast cancer is the most common cancer type in women, with an estimated 1.3 million cases annually. Therapies conventionally used for breast cancer include surgical removal, radiotherapy, and chemotherapy. However, these treatment modalities are harmful to healthy cells, causing undesirable side effects. Therefore, the seeking for alternative treatments that can reduce side effects is of extreme clinical importance. Antimicrobial peptides (AMPs) may represent an alternative that can be used alone or combined with other drugs already used in breast cancer treatment. AMPs are small molecules (5 up to 100 aa) with a broad spectrum of targets. In addition to eliminating neoplastic cells, binding to their plasma membrane, and forming pores alongside the membrane, AMPs also interact with intracellular targets, changing the gene expression profile of these cells. The RNA-seq transcriptome analysis of cells exposed to peptides with potential antitumor activity is a method that allows us to identify genes and/or gene networks that may be related to the emergence, maintenance, proliferation, and invasion of cancer. Therefore, the present work aims to evaluate the transcriptome of breast cancer strains exposed to peptides (with an antitumor potential) to find possible molecular markers related to the development of this neoplasm.

A1.R8 - In vitro effects of omega-3 (DHA) stimulus on murine melanoma cells B16F10

Ramon Buson Lima Paiva[1]; Débora Santos da Silva[1]; Heloísa Antoniella Braz De Melo[1]; Luana Borges Baptista[1]; Kelly Grace Magalhães[1]

[1]Laboratório de Imunologia e Inflamação – Universidade de Brasília (UnB)

Omega-3 polyunsaturated fatty acids (n:3 PUFAS) have clinical relevance due to their protective mechanisms against the establishment of several diseases, including tumors. The antitumor actions of this lipid include: prevention of carcinogenesis, induction of cell death, modulation of the microenvironment, inhibition of angiogenesis, among others. Melanoma, is the most aggressive form of skin cancer, due to its high metastatic capacity. In this context, this work aims to identify possible mechanisms of action of omega-3 in the treatment of cells derived from murine melanoma. Methodology: Murine metastatic melanoma B16F10 cells were stimulated (or

not) with docosahexaenoic acid (DHA) for 24 or 48 hours at a concentration of 12.5, 25, 50µM. Cell viability was assessed by MTT a; Cell proliferation was assessed by CFSE and analyzed by flow cytometry. Pore formation was evaluated by staining cells with propidium iodide and analyzed by spectrophotometry. Lipid droplet biogenesis was assessed by Bodipy staining and analyzed by flow cytometry. Results: We observed a dose and time dependent decrease in cell viability of melanoma cells induced by DHA treatment. Moreover, DHA was also able to induce pore formation in the membrane of metastatic melanoma. In addition, we also observed a significant decrease in cell proliferation after treatment with this molecule. However, lipid droplet biogenesis was not modulated by DHA. Conclusion: Our data support the antitumoral effects of DHA, considering the reduction in cell viability, induction a lytic death and the decrease in cell proliferation, demonstrating a potential to interfere with the tumor cell cycle progression. Thus, this work demonstrates new perspectives on the use of omega-3 as an adjuvant treatment in the treatment of melanoma skin cancer.

A1.R9 - The role of omega-3 in white and brown adipose tissue modulation: differential functions involved in the progression of metastatic melanoma

Débora Santos Silva[1]; Heloísa A. Braz-de-Melo[1]; Luana B. Baptista[2]; Ramon B. L. Paiva[2]; Kelly Grace Magalhães[1] .
[1]Laboratory of Immunology and Inflammation, Department of Cell Biology, University of Brasília, Brasília, DF, Brazil

Omega-3 is a class of essential polyunsaturated fatty acids. Among the main representatives of omega-3, the docosahexaenoic acid (DHA) is able to restore the body's homeostasis, in addition to providing protection in different comorbidities, in which obesity stands out, since it has a direct action on adipose tissue. This tissue is characterized as an endocrine organ, with high phenotypic and metabolic plasticity, mainly composed of white and brown adipocytes, which play differential roles in the progression of different pathologies, especially melanoma. Taking into account the distinct role of these adipocytes in the tumor microenvironment, as well as possible metabolic alterations induced by omega-3, this work aims to analyze the modulatory potential of DHA on white adipose tissue (WAT) and brown adipose tissue(BAT) and their function on the melanoma. Mice(C57/BL6) supplemented or not with DHA at a concentration of 1g/kg for 30 days were used. After this period, body weight, gonadal white adipose tissue(gWAT) and subcutaneous tissue(sWAT), BAT, liver and spleen were weighed. We also performed the cytokine quantification of the serum. The peritoneal lavage cells were stained to verify the lipid droplets(LD) biogenesis and reactive oxygen species(ROS) formation. In addiction, we exposed the melanoma cells(B16F10) to the secretion product of BAT and WAT, and evalueted carcinogenic parameter such as cell viability and cell death. Our data demonstrated that DHA reduces the weight of gWAT and sWAT of the supplemented animals, as well as reduces LD biogenesis, ROS formation of the peritoneal lavagem and serum cytokine levels. In addition, the stimulation of B16F10 with the secretion products of BAT supplement animals led to a decrease of cell viability as well as increased cell death. Thus, this work demonstrates the potential of omega-3 to modulate adipose tissues and generate new perspectives for the use of secretion products as adjuvants in the treatment of melanoma.

A1.R10 - Evaluation of bone loss prevention by nanostructured doxorubicin

Marina Arantes Radicchi [1,2]; João Paulo Figueiró Longo [2]; Sônia Nair Báo [1].
[1]Laboratório de Microscopia e Microanálise; [2]Laboratório de Nanobiotecnologia; UnB.

Breast cancer metastasis implicates in multiple hospitalizations with health conditions associated with chemotherapy as osteoporosis and bone loss, cardiopathy, respiratory failure1-3. Doxorubicin is widely used as a chemotherapy agent for solid tumors, such as breast cancer, and its pharmacodynamics includes oxidative stress,

metabolism modulation, pro-inflammatory stress and DNA intercalation2. Pharmacokinetics modulation due nanoestruturing of doxorubicin can overcome the problems cited above. Solid lipid nanoparticles (SLN) containing doxorubicin (SLNDoxFB) were formulated by high energy and high temperature method and particles were stable on different storage conditions up to one month. For morphology purposes, transmission electron microscopy was performed and SLN were spherical with gaussian size distribution (Figure 1). For protocol determination, a pilot experiment was performed with long bones collected from balb/c mice, these bones were exposed to doxorubicin and SLNDox, after the treatment, computed tomography was used to determine the bone loss. In addition to ex vivo experimentation, the in vitro assay is being performed with pre-osteoclast cell lineage, at the same time, the in vivo tests are starting. With this experimental design, we expect to observe the prevention of bone loss associated with breast cancer due to different pharmacokinetics from SLNDox in comparison to commercial doxorubicin.

A1.R11 - Analysis of the impact of SARS-CoV-2 Nucleocapsid protein on cell metabolism and inflammatory profile of white and brown preadipocytes

Gabriel Felipe Gomes Calixto[1]; Raquel das Neves Nascimento[1]; Milena Nascimento Verdam de Araujo[1]; Kelly Grace Magalhães[1].
[1]Laboratório de Imunologia e Inflamação – Universidade de Brasília (UnB)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus belonging to the Coronaviridae family that has impacted public health worldwide. SARS-CoV-2 infection can lead to several signs and symptoms, but certain groups affected by the infection may develop a more severe condition, such as in cases of obesity. Obesity is classified as an epidemic and is associated to alterations in adipose tissue (AT) composition and endocrine function. This organ is mainly composed by white adipocytes, which are related to energetic storage and secretion of a range of endocrine factors, or by brown adipocytes, which has a central role on energy expenditure, especially through thermogenesis. It has been widely discussed the relationship between AT and viral infectivity in several disorders. Nevertheless, it is poorly understood whether SARS-COV-2 is able to directly modulate AT homeostasis. Considering this, the present project aims to evaluate whether the N protein of SARS-CoV-2 directly impacts on brown (9-BAT) and white (9-WAT) pre-adipocytes, focusing on cellular metabolism and inflammatory profile, aiming to investigate a possible specific tissue effect, which may impact in the context of obesity and metabolic syndromes. Our results showed that SARS-CoV-2 N protein was able to reduce the cellular viability of both 9-WATand 9-BAT. It was also observed a reduction of TGF-β secretion in both cells, which would lead to a more pro-inflammatory microenvironment. Moreover, it was observed a reduction of IL-33 in 9-BAT cells. In addition, an increased lipid droplet biogenesis was observed in white, but not in brown pre-adipocytes. SARS-CoV-2 N protein also led to a decrease in reactive oxygen species in both pre-adipocytes cells. Taken together, these data demonstrate important clues regarding an inflammatory impact of SARS-CoV-2 on AT microenvironment and enabling new perspectives of the impact of this pandemic viral on brown and white adipose tissue homeostasis.

A1.R12 - Cytotoxicity assay of lipid nanoparticles associated with docetaxel in gastric adenocarcinoma

Laís Vaz-Costa; Sônia Nair Báo.
Universidade de Brasília

Stomach cancer is the most common neoplasm that affects the digestive system and can result from multiple factors, usually caused by interactions between the environment and genetic factors. Docetaxel (DTX), one of the most used drugs for the treatment of adenocarcinomas, has a mechanism of action in inhibiting mitosis and cell division, which can cause hypersensitivity, nephrotoxicity and fluid retention . However, their encapsulation in solid

lipid nanoparticles (SLN) may reduce these problems, improving their effectiveness and carrying out the drug directly to interact with tumor-specific cells. This work objective to evaluate the cytotoxic effect of the formulation of SLN associated with DTX in gastric adenocarcinoma (AGS) in vitro. The cytotoxic action on the AGS was estimating with four treatment groups were performed: (1) SLN-DTX containing 1 mg/mL of DTX; (2) SLN; (3) DTX and (4) ethanol. Cell viability test, morphology and cytoskeleton analysis, colony formation assay, cell death, cell cycle, protein expression, cytokine and cell proliferation assays were performed. With the results obtained through the MTT assay, it was possible to observe that SLN-DTX have cytotoxic activity from 10 ng/mL and DTX from 10 µg/mL in 24 hours; after 48 hours there is a reduction of more than 50% in both treatments, suggesting a dose and time-dependent effect. Through the morphology tests, it was possible to notice that the cells increased their volume and decreased the cytoplasmic projections after the treatment. The SLN-DTX and DTX induced damage to the microtubules resulting in a stop of the cell cycle in G2/M resulting in 73.5% and 66.5% of death by apoptosis, respectively. Therefore, with the results of this work, it was possible to conclude that the association of DTX with SLN proved to be efficient, presenting cytotoxic action in gastric adenocarcinoma cells, favoring the use of this formulation in the administration of medications.

A1.R13 - The role of Omega-3 DHA/EPA on establishment and development of orthotopic mice breast cancer

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Polyunsaturated fatty acids (PUFA) correspond to a class of essential lipids for the organism, amongst them, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) exert main biological effects, mostly as anti-inflammatory precursors. Notably, PUFAs can affect immune function, gut microbiota and the development of some tumors. Our group has previously demonstrated that omega-3 DHA has an antitumor effect against breast cancer in vitro. In this present work, we aimed to evaluate the modulatory effects of omega-3 supplementation on breast cancer development in mice. Balb/C mice were supplemented or not with two DHA doses, 500 mg/Kg and 2g/Kg, for 60 days and inoculated or not with 4T1 breast cancer cells on the 30th day of supplementation. Tumor size and animal weight were measured throughout the process. Euthanasia was proceeded on the 60th day, followed by blood collection in order to perform hematological, biochemical and cytokine dosage analysis. Our data showed that the under higher omega-3 dose supplementation mice showed decreased levels of aspartate transaminase, alanine aminotransferase, cholesterol and high-density lipoprotein. inflammatory and carcinogenic parameters are still under investigation. Thus, this work is still under development and aggregates data about the potential of PUFAs in mitigating breast cancer development.

A1.R14 - Characterization of Vitamin E effects on carcinogenic and neuroinflammatory parameters in neuroblastoma

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Neuroblastoma is a pediatric extracranial tumor, that occurs in sympathetic nervous system tissues. It is the most frequent cancer in the first year of life. Carcinogenic factors that are involved in tumor growth and progression are sustained inflammation, immunosuppression, cell death inhibition, enhanced proliferation and angiogenesis. In this scenario pyroptosis plays an important role. In contrast with a silent cell death (apoptosis, mediated by caspase

3), pyroptosis is an inflammatory and lytic cell death, mediated by inflammasome. Caspase-1 and Gasdermin D (GSDMD) are essential proteins in inflammasome activation and membrane pore formation, secreting IL-1β and IL-18, accounting for recruitment of immune cells. Molecules with the capability of modulating these parameters are potential adjuvants for the current treatments. Vitamin E as an antioxidant is of interest since tumor cells produce high amounts of reactive oxygen species (ROS). It maintains membrane stability and prevents oxidative stress. Studies demonstrated a reduction of viability in prostate and breast cancer cells. It is plausible to consider it may have similar effect in neuroblastoma. This project aims to analyze in vitro effects of Vitamin E in carcinogenic and inflammatory parameters (viability, proliferation, cell cycle, cell death, angiogenesis, eicosanoids and cytokines). Neuroblastoma cell lineages (SK-N-BE(2); SH-SY5Y) will be treated with mixed tocopherols in four concentrations (10, 20, 30, 40μM) for different times (3, 12, 24, 48, 72h). We will assess cell viability with MTT assay and cell proliferation with CFSE staining. Cell death will be assessed by Annexin-V and PI staining, pore formation assay, lactate dehydrogenase (LDH) secretion, translocation of HMGB1 and Western-blotting (Bax, Bcl-2, cleaved caspase 3, caspase-1, GSMD). VEGF, IL-1β, IL-6, TNF-α, IL-4, IL-10 and TGF-β will be detected by ELISA. Eicosanoids will be measured with a competitive immunoenzimatic assay (EIA).

A1.R15 - Production of a cancer marker protein using the *Cell-free transcription-translation technique*

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Synthetic Biology is a branch of Biology that unites several other areas of knowledge, with the aim of producing proteins and other cellular compounds through “Cell free” techniques, that is, without the use of cells. Through these techniques it is possible to produce proteins with a high degree of yield and purity, without high costs or extremely qualified professionals. GAGE antigens comprise a family of 16 genes located in tandem on the short arm of the X chromosome. These antigens, also known as cancer/testis (CT) antigens, are detected in many types of cancer. Therefore, these proteins have been studied as candidates for anti-mural immunotherapy. Within the GAGE family, the GAGE-1 gene has been described as encoding an antigenic peptide called YRPRPRRY, which was previously identified in MZ2-MEL human melanoma cells and recognized by cytotoxic T lymphocytes originating from the tumor patient. The main objective of this research is to express through the technique “Cell-free transcription-translation” (TXTL). The GAGE-1 protein was codon optimized using online IDT software for its expression in Escherichia coli. The plasmid was synthetically constructed in the pHis2008p vector with histidine tail in the C-terminal portion. Plasmids were cloned to obtain DNA in E. coli DH10B and stored at -20°C. For the expression of proteins in the Cell-free system, several expression optimization parameters were tested, such as reaction incubation temperature, DNA concentration and reaction volume. Our results demonstrated that using a concentration of 30nM of DNA, the GAGE1 protein is produced at an incubation temperature greater than 25° C in a volume of 25 uL. The kinetics results revealed that the GAGE protein is produced after 3 hours of incubation, continuing its production in up to 48 hours. For this purpose, its expression was standardized in volumes greater than 25 uL at 29° C for 12 hours. Other experiments will be carried out to test the immunogenicity and cytotoxicity of this protein.

A1.R16 - β-glucan modulation of the gut microbiome and its influence on colitis induction

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Humans and microorganism live in harmony, one of the most intricate interactions that exists are the ecossytems of microorganism living in the human host, in special the gut microbiota, which composition depends on multiple factors like, life style, food consumption, hygene, and many others. The gut microbiome is a complex ecosystem in which, bacteria, fungi, virus, archea and others live symbiotically with the human host, this equilibrium occurs throught the complex interactions between microorganism and the constant vigilance of the immune response and its modulation, in which the immune cells modulate and are modulated by the microorganism. Auricularia auricula is a fugus of the basidiomycetes class, it secretes 1,3 betaglucan, which can be extracted. This type of polysaccharide is recognized by the dectin 1 receptors and there are studies showing the modulatory capacities of these substance working as an anti-inflammatory and as a pro-inflammmatory compound. A murine model was chosen to study the modulation capacity of these polysaccharides. C57 WT and dectin 1 KO mice will eat 1,3 betaglucan purified from A. auricula and colitis will be induced by dextran sulfate sodium (DSS) ingestion, then the tissue will be removed and analyzed for the pathological score, and for gut RNA quantification. Also the population of microbiota will be analyzed to detect changes in diversity before, during and at the end of experiment, detection inflammatory cytokines will be done by ELISA. The objective of this study is to evaluate the capacity of the 1,3 β-glucane to modulate the intestinal imune system and modifing the existing microbiome.

2. Bioquímica, biofísica e biologia estrutural (A2)

A2.R1 - Characterization of enzymatic parameters of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* co-cultures

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Fungal co-cultures are a new and yet unexplored way of producing enzymes. Such enzymes are used in biorefineries to produce a myriad of sustainable products from agro-industrial waste. *Trichoderma reesei* RUT-C30 and *Aspergillus niger* were tested as producers of carbohydrate active enzymes in both mono and co-culture, using sugarcane bagasse as the carbon source. The effect of fungal inoculation order was also assessed. Results show that both fungi can produce enzymes, and the production profiles vary according to culture parameters. Xylanase activity after de 4th day was very similar for all conditions. The co-cultures of *A. niger* + *T. reesei* RUT-C30 with delays of 48h and 24h obtained β-xylosidase activities 96% (48h) and 56% (24h) greater than simultaneous cultivation (0h); The effect of temperature in enzyme activity also varied greatly depending on the culture conditions. Pectinase activity at 50 °C showed the most variation with 20% difference between the monoculture of *T. reesei* and the co-culture of *T. reesei* and *A. niger* with 24h and 48h delays. Xylanase showed similar activities among all culture conditions regardless of the temperature. The results shows that co-culture is a viable option for carbohydrate-active enzyme production. However, co-cultures are not yet optimized for industrial production of such enzymes are very little is known about the fungal interactions occurring in the co-culture. This lack of knowledge hinders the rational design of culture conditions to improve enzyme production.

A2.R2 - Diagnostic method for SARS-CoV-2 based on detection of viral RNA by Ribozymes associated with DNA-hairpins in a hybridization chain reaction with fluorescence resonant energy transfer

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COVID-19 is a disease caused by the SARS-CoV-2 virus. There have been more than 246 million people infected and has already caused more than 4 million deaths worldwide. Brazil is the second country with the largest number of people infected and killed by this virus. The current COVID-19 diagnostic method for the initial phase of the infection is the RT-qPCR.This gold standard method, however, has several limitations, such as the requirement for skilled labor, high cost equipment and reagents, considerable execution time and laborious procedures. In this sense, our group proposed to develop a SARS-CoV-2 diagnostic method that is as efficient and fast as the gold standard, but with a lower cost, no requirement of equipment and skilled labor and free of enzyme reaction. The proposed method consists of three steps. The first one is the recognition and cleavage of the extracted viral RNA by two designed ribozymes. The second step consists of complexing the released viral fragment with two different metastable DNA hairpins, containing different fluorophores: one as a donor, and the other, an acceptor, composing a FRET system. The third step is characterized by the increase of acceptors fluorescence emission due to the polymerization of the probes. The genomic targets for the ribozymes consisted of consensus SARS-CoV-2 sequences identified from the alignment of the genomes available in databases. Three ribozymes were designed using RiboSoft software and their activities were evaluated according to the protocol described by Kharma et al., 2016, with preliminary results showing one functional molecule. Then, two DNA hairpin reporters molecules were selected using NUPACK software, however FRET reaction was not detected even on the positive control, in which a target DNA molecule was used. Relying on parameters defined on reference articles, new DNA harpins molecules were designed and will be evaluated following the DBTL cycle preconized by the Synthetic Biology research area.

A2.R3 - Structural and electrophysiological evaluation of Nav channel modulators isolated from *Tityus stigmurus* venom

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Scorpion venoms are known as rich mixture of components, including nucleotides, lipids, biogenic amines, peptides and proteins. Among peptides are those able to interact with different ion channels, particularly Na+, K+, and Ca2+ channels, essential membrane proteins for various physiological functions in organisms. The present work aimed to identify peptides from *Tityus stigmurus* venom that are capable of interfering on Na+-channel activity. The venom was extracted through electrical stimuli and subsequently fractionated using reverse phase high-performance liquid chromatography (RP-HPLC). The molecular masses (MW) of the peptides found in the most abundant fractions were evaluated by mass spectrometry (MALDI-TOF). Fractions contained 6-8kDa peptides, were further fractioned to obtain purified peptides. NaTx had the amino acid sequence obtained by the ISD (In Source Decay) technique. Electrophysiological studies are being made using whole-cell patch-clamp. Venom fractioning resulted in 48 fractions, whose 15 were submitted to mass spectrometry analysis. Focusing on NaTx, two peptides were isolated, and the MW obtained were 7406.8 Da and 6981.8 Da. Both toxins were partially sequenced, 37 and 21 amino acids were identified, respectively. The aim of the present study is to evaluate their electrophysiological activities on

Nav channels subtypes. Until now, Tst1 showed an activity on Nav 1.2 and 1.5 leading both channels to work in a hyperpolarization state, and open in lower potentials, being more active on Nav 1.2. These results demonstrate that Tst1 is probably a β-toxin. Tst3 was capable to modify the inactivation of Nav 1.5 channel, increasing the sodium influx in the cell and maintaining the channels depolarized for longer time, which affects the kinetics of the channel and the cell. Data obtained with Tst3 indicate that this toxin is probably an α-toxin. Assays with other Nav subtypes are being performed to describe their complete activity.

A2.R4 - Expression and Purification of SARS-CoV-2 Main Protease (Mpro)

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The Covid-19 pandemic is caused by the SARS-CoV-2 virus and has led to more than 5 million deaths worldwide. Despite successful global vaccination efforts, the risk of outbreaks by resistant variants and the lack of effective antiviral therapeutics demand more selective pharmacological treatments, such as those based on viral-protease inhibitors. The chymotrypsin-like Main Protease of SARS-CoV-2 (Mpro, also named 3CLpro) is a highly conserved and attractive antiviral target due to its essential function in proteolytic processing of the two pp1a and pp1ab polyproteins during viral replication. The black-eyed pea trypsin and chymotrypsin inhibitor (BTCI) is a small protein belonging to the Bowman-Birk family isolated from Vigna unguiculata seeds that bifunctionally inhibits trypsin- and chymotrypsin-like proteases. Peptides that mimic BTCI reactive loops retain their inhibitory capabilities. Molecular docking with these peptides and BTCI within the Mpro substrate-binding pocket indicated a strong binding affinity, indicating the inhibitory potential of BTCI and derived peptides against this SARS-CoV-2 protease. In order to elucidate Mpro inhibition mechanisms by BTCI and related peptides, multiple molecular biophysics techniques and enzymatic assays will be performed. In order to obtain Mpro, E. coli BL21(DE3), XL-10 Gold and E. coli BL21(DE3)pLysS cells were transformed with pGEX-6P1 plasmid that encodes the fusion GST-Mpro-6xHis protein cleavable by PreScission protease for removal of GST- and 6xHis-tags. Expression assessment indicated low Mpro expression levels in all cells used, but E. coli BL21(DE3) showed better cell growth. Expression was confirmed by western blotting, and the supernatant of the lysed cells was applied to Ni-Affinity for fusion protein purification. Cleavage by PreScission, combined with GST-glutathione and Ni-Affinity chromatography should yield Mpro without tags and higher purity.

A2.R5 - Construction and characterization of serine proteases with novel catalytic triads

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Serine proteases are the most abundant group of proteolytic enzymes, being of great importance to cellular and viral physiologic processes, as well as being widely used in textile, pharmaceutical, and food industry. Their action is based in three catalytic and structural components: the catalytic triad, the oxyanion hole, and the specificity pockets. The catalytic triad residues are arranged in the primary protein structure in four different sequence orders in the classical serine protease clans, indicating at least four different evolutionary origins of this machinery and allowing separation of these proteases according to their triad arrangement. However, in theory, the triad residues can be arranged 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 triad orders, produce these

enzymes and perform their biophysical and biochemical characterization. The first step is to separate the structures already deposited in the Protein Data Bank (PDB) according to the order of their catalytic triad. The distance between the catalytic residues will be used as criterion to locate serine proteases because, despite the different triad arrangements, the geometry of the active site is conserved. For this, an algorithm that calculates the distance between specific residues was developed using the Python language and biopython tools. The program was used to calculate the distance between catalytic residues of several known serine protease structures to establish a cut-off distance value that will be used in future searches for serine proteases in the PDB database. The enzymes found will be classified according to the triad arrangement present in them and used in the design and construction of novel enzymes, which will be expressed and characterized

A2.R6 - Kinetic and structural study of the interaction between ferulic acid and HXYN2 endoxylanase from *Humicola grisea* var. *thermoidea*

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Recent studies report the effect of phenolic compounds derived from lignin on fungal xylanases, inhibiting and/or deactivating these enzymes. Other studies describe the activating effect of some of these compounds, such as ferulic acid (FA), on the enzymatic activity of xylanases. This work aimed to elucidate the mechanisms involved in the activation of recombinant HXYN2 from *Humicola grisea* var. *thermoidea* by FA, based on Michaelis-Menten kinetics, conformational changes by Fluorescence spectroscopy and secondary structure content by Circular Dichroism, in the presence of this compound. The Michaelis Menten kinetic parameters of HXYN2 were different with AF at different concentrations. At concentrations of 0.5 and 1 mg/mL, an increase in Vmax, Kcat and catalytic efficiency was observed, while Km was maintained. In the presence of FA, the fluorescence spectra of HXYN2 showed emission band displacements and the changes in fluorescence intensity, dependent of pH. Fluorescence quenching data suggest that FA interacts in a pH-dependent manner with solvent-exposed tryptophan residues showing binding constant values of 5.93 x 108, 2.40 x 105 and 8.92 x 105, indicating moderate to strong affinity between the two molecules. The secondary structure of HXYN2 in the presence of FA was changed by decreasing the α-helix and increasing the β-turn and random coil contents. The thermal denaturation curve showed a Tm of 54 °C, indicating lower structural stability of HXYN2 in the presence of this compound. The interaction of HXYN2 with FA was investigated by isothermal titration calorimetry and molecular docking and are part of an article, to be submitted. Altogether, the results showed that FA promoted an increase in the catalytic efficiency of HXYN2 by conformational and structural changes without affecting the affinity of the enzyme to the substrate.

A2.R7 - Study of serine proteases evolution through ancestral sequence reconstruction technique

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Serine proteases are enzymes that use serine residues in it's active site in order to perform protein hydrolysis. They are present in eukaryotes and prokaryotes and can be classified by it's structure, considering the ones with trypsin and chymotrypsin- like folding the most studied. In metazoa, trypsin-like folding is found in multiple-classes evolutionarily related proteases which are also involved in several biological processes, such as kallikreins, trypsins and SVSPs (Snake Venom Serine Proteinases). The divergent evolution that resulted in such

distinction can be studied through ancestral sequence reconstruction technique. In this approach, ancestral sequences are built from modern sequences, enabling us to better understand enzymatic and structural properties of extinct proteases. Therefore, the goal of this work is to study the structure evolution of metazoans' serine proteases (particularly the ones present in Squamata venom) by using the biochemical and biophysical characterization of ancestral enzymes. Only the metazoan sequences were obtained by using the HMMER tool against MEROPS database. Next, they were clustered (90, 60 and 30% of identity) by using the CD-HIT software, having only the cluster containing trypsins (which was reclustered in 80% and 90%) for further studies. We built phylogenetic trees with final sequences by using the MEGA-X software (ML method). The trees revealed three related protease classes: kallikreins, trypsins and SVSPs. The SVSPs clade was selected for further study regarding its evolution. The ancestral soon to be reconstructed are being chosen at this moment. Once the sequences are obtained, their genes will be synthesided, expressed and the enzymes structurally characterized. With this study we hope to expand the knowledge in functional and structural evolution of proteases.

A2.R8 - The importance of scientific dissemination for Diabetes Mellitus education

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The prevalence of Diabetes Mellitus (DM) has been rising worldwide, and it has become a severe public health problem. Also, DM is associated with several risk factors, including obesity. Type 2 DM (DM2) is the most prevalent DM form, associated with many complications. In some cases, the complications may develop as the DM2 diagnosis is commonly delayed. Our work aimed to inform the population about DM by scientific dissemination in social media, universalizing scientific knowledge. We performed bibliographic research to gather relevant epidemiological data, articles, and reviews published in indexed journals and organizations. The collected data were critically evaluated to produce texts in accessible language to no-academic readers. Thus, we created artistic posts to publicize at BEM lab social media and evaluated them using conventional social media tools and metrics. Herein, we report the results obtained by five posts. The posts were produced with an average of 6 pages each, from January 26 to February 26, 2021. On Instagram, they reached an average of 282 people and about 393 reads or impressions, with the post about DM2 having the best results (309 people reached). On Facebook, an average of 60 people were reached per post. The post we published first on Facebook was the most accessed, probably because it showed the main DM2 characteristics and their association to COVID-19. The emergence of Fake News leads to misinformation and puts the population's health at risk. In addition, incorrect DM self-care may worsen the DM comorbidities and the DM itself. Thus, we believe that scientific dissemination of DM, containing correct information and simple language, is critical for improving patients' quality of life.

A2.R9 - Scientific divulgation on the relationship between obesity and cognitive decline

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Obesity, a worldwide public health problem, is characterized by an excessive accumulation of fat due to positive energy balance, leading to chronic diseases. As life expectancy increases, the association between obesity and neurocognitive diseases has become more evident. This study aimed to conduct bibliographic research about the association between obesity and cognitive dysfunction. The articles found were then used to create social media posts in an accessible language to reach the general public, promoting health education. A bibliographic review was performed by searching and epidemiological data on the relationship between obesity and cognitive decline. Data were acquired from 6 original articles and reviews published in internationally recognized journals, available in databases such as Pubmed, LILACS, ScienceDirect, and Scielo. In addition, nationally or internationally recognized data platforms were used as sources, such as the World Health Organization (WHO) and the Alzheimer's Association. The result of the work was the production of 2 posts connecting obesity and memory. This material showed the public that the development of cognitive decline could be a consequence of obesity. Diet-induced cognitive changes may alter, anatomically and functionally, the brain. Also, we created content referring to a recent laboratory article, which explored how a high-fat diet impacts zebrafish brains. The Instagram post reached 305 accounts and received 421 impressions, while on Facebook, 198 accounts were reached. The post linking the animal model, obesity, and memory reached 378 accounts on Instagram and 714 on Facebook, with 525 impressions on Instagram. As the prevalence of obesity is increasing and an expressive portion of the population is still unaware of its complications, our divulgation materials were prepared to inform about the relationship between obesity and cognitive decline.

A2.R10 - Social media as a vehicle to raise awareness about the double burden of malnutrition

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Malnutrition is a global concern targeted in the United Nations sustainable development goals, whose main priorities are ending all forms of undernutrition and hunger and ensuring access by all people to safe, nutritious, and sufficient food until 2030. Unfortunately, there are more than 149 million children worldwide with stunted growth, and, on the other hand, childhood obesity has been growing globally. Thus, the scientific community still has much work to understand and combat malnutrition, which has historically been studied through two opposing perspectives, focusing on undernutrition or obesity. Nevertheless, many individuals deal with the double burden of malnutrition when they are simultaneously overweight and malnourished. This scenario is alarming since these conditions can cause serious long-term health consequences. In light of the need to communicate scientific-based data regarding these challenges in an accessible language to a broad public, this study aims to use social media platforms to raise awareness regarding malnutrition, paying particular attention to the double burden of malnutrition. Therefore, we conducted a bibliographic review in databases such as Pubmed and Scielo to find scientific articles that would become the primary references for the content developed. From the 213 papers found, 11 were selected to support three posts about obesity, undernutrition, and the double burden of malnutrition. The

posts were shared on @labdebem's accounts on Facebook, Instagram, and Twitter. These three posts received 57±4,70 likes and reached 302±3,48 accounts on Instagram per post. In conclusion, this study outlined how effective science communication can be a solid ally to combat malnutrition.

A2.R11 - Electrophysiological characterization of the L1650P and L1660T mutations in the SCN2A gene associated with epilepsy and the antiepileptic evaluation of the Tst2 peptide from the scorpion *Tityus stigmurus*

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Epilepsy is a disease caused by exaggerated activity of neurons or abnormal brain synchrony. It can be focal, generalized, or unknown. Infections, autoimmune diseases, acquired causes, and genetic mutations are the main causes. Among those caused by mutations, we can highlight mutations in voltage-gated ion channels. Among the mutations in the SCN2A gene (hNav1.2), several studies have been carried out with epilepsy. Trump et al. (2016) analyzed 400 patients with early-onset seizures and/or severe developmental delay. In the middle of the analyses, a mutation in the SCN2A gene (L1650P) was described. This mutation is related to early childhood epileptic encephalopathy. Furthermore, Fukasawa et al. (2015) described a case study reporting a young male with recurrent acute encephalopathy presenting with repetitive generalized tonic-clonic seizures and diffuse brain atrophy. That said, genetic analyzes were performed and the presence of the L1660T mutation was observed. Navs present an important binding site for antiepileptic drugs. However, part of the population with epilepsy is resistant to the action of drugs. It is necessary to search for new ways of treating the disease, with natural products, such as scorpion venom. The objective of the project is to electrophysiologically characterize the L1650P and L1660T mutations in the Nav1.2 channel and to evaluate the antiepileptic potential of the Tst2 peptide isolated from the *Tityus stigmurus* scorpion. The purification and identification of the peptide was done by HPLC and MALDI-TOF. The mutation will be performed by the directed mutagenesis system. Electrophysiological recordings will be made by Patch clamp in whole-cell mode. Currently, the purification of the peptide (Tst2) and the identification of its experimental mass in linear (6991,729 Da) and reflective (6993,149 Da) mode were obtained. Next steps: partial sequencing of the peptide, carrying out mutations in the channels, and analyzing electrophysiologically.

A2.R12 - Sponges and the possible evolutionary origin of POS

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Many animals endure seasonal environment changes that might lead to oxidative stress. Some examples are hypoxia and aerial exposure, when low concentrations of oxygen or the inability to extract oxygen cause hypoxia. Some animals evolved a biochemical adaptation that is especially important at reoxygenation. The "Preparation for Oxidative Stress"(POS), has been observed in 9 different phylum and elucidates that an upregulation of endogenous antioxidant defenses during hypoxia minimize the oxidative stress when oxygen availability is restored in the habitat of these animals. The Porifera form a basal phylum and is a potential model for studies on the ecophysiology of biochemical adaptations, like POS. Fossil records suggest there were living sponges 890 million years ago, when oxygen concentration was very low. These animals probably developed some mechanism to survive this condition, and POS is a possibility. This research's hypothesis is that POS may have originated in ancestral sponges. To test that, the chosen model was *Hymeniacidon heliophila*, mostly found in coastal environments, where the sea tide rises and falls everyday. Even with daily aerial exposure the sponge survives.

Animals were sampled at São Sebastião/SP and separated in 4 groups, based on two stressors: aerial exposure and UV radiation. The samples will be used to analyze antioxidant enzymes, lipid peroxidation, glutathione (GSH) and total antioxidant capacity. Some of the expected results are: an up regulation in enzymes and GSH during air exposure, increase in oxidative damage in submerged sponges, and general increase in oxidative stress during the day. The research does not have any biochemical data yet. The results could prove that sponges can do POS in nature. More than that, POS could have appeared more than 800 million years ago, maybe from inheritance of ancestral sponges. If this proves to be true, it would bring for the first time the evolutionary origin of POS.

A2.R13 - Molecular characterization of medicinal plant-based nanostructures by Raman spectroscopy

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Nanostructures with amphipathic constituents present numerous advantages over other nanomaterials and one of them is that they can carry both hydrophilic (such as aqueous extracts) and hydrophobic (such as apolar compounds) actives. Raman spectroscopy allows distinguishing chemical groups present in nanostructures when they contain only the dispersant (water) or some plant extract; and even infers whether some spontaneously formed nanostructures corresponds to liposomes or other molecular array. For this purpose, nanostructures containing water or extract were deposited separately onto a stainless-steel microplate with 20 successive 1 µL applications in each spot. Analyses were performed using a Raman Alpha 300 RA confocal microscope. Nanostructure samples containing *Pelargonium graveolens* extract or containing water (control) were analyzed separately. At least 8 distinct regions in each sample were analyzed. Raman spectra of nanostructures produced using an aqueous extract of *P. graveolens* demonstrate the presence of different peaks/bands corresponding to the functional groups present in the aqueous phyto-extract. The results show that the signals corresponding to chemical groups of phyto-extract molecules suppressed those associated with phospholipids used for nanostructures formation. When evaluating the peaks attributed in the Raman spectrum of nanostructures formed from lipid films resuspended in water only, the presence of a peak that occurs in micelles and liposomes was observed. This signal normally does not change, even if the content conveyed in the nanostructures is varied, since it refers to vibrations located within the hydrophobic portion of lipid bilayers and lamellar structure. It is concluded that the evaluation by Raman spectroscopy allowed to confirm that the nanostructures were liposomes due to the indication of the signals corresponding to lipids in the spectrum.

A2.R14 - Resolution of artificial protein-protein interactions from optimized sequence landscapes

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Coevolutionary theories describe the probability distribution of interacting proteins in terms of a Boltzmann statistical model - with Hamiltonian given by a set of local fields and statistical couplings. 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 interolog systems in general, their sequence distributions may have not been fully shaped by selective pressures opening the possibility that novel interolog sequences could be selected from artificially generated lower entropy distributions. That artificial process would in principle allow for designing of a new subset of likely sequences. The

goal of this work was to artificially select a distribution of non-native sequences with fixed composition and evaluate their physical meaning. For that, we explore a Genetic Algorithm (GA), which solves the distributions by maximizing the statistical coupling, starting from the native multi-sequence alignments (MSA) and exploring the space of scrambled MSAs. Once likely non-native sequences are selected from optimized distributions, their binding free-energies at a fixed native bound state are evaluated according to MMPBSA calculations. We have not yet obtained the convergence of the GA, but using an intermediate individual from this simulation it was already possible to obtain a distribution with lower entropy relative to native. Maximized MSA has the same composition when compared to native, but has higher statistical coupling values and therefore, more probably sequences. The same is not observed for a random scrambled MSA, which has lower values than the native. We also noticed a significant difference between the binding free-energies of native sequences when compared to random sequences, but calculations for maximized sequences still depend on GA completion.

A2.R15 - Gene and molecular prospection focusing on resistance to *Pseudocercospora musae* and tolerance to drought stress in *Musa acuminata* subsp. *burmannicoides*, var. Calcutta 4

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Banana (*Musa* spp.) is an important fruit crop in more than 130 countries. Although wild species are fertile, commercial varieties are often sterile, with limited genetic variation resulting in susceptibility to phytosanitary problems. Yellow Sigatoka disease, caused by *Pseudocercospora musae*, is a major problem in banana, resulting in reduced functional leaf photosynthetic area and production losses of up to 50%. Drought conditions are a serious obstacle to the growth of global agriculture. Banana is a hydrophytic plant, considered sensitive to water deficit and responsive to irrigation, such that water is the most limiting abiotic factor for its production. The objectives here were to identify genes involved in both resistance to *P. musae* and tolerance to drought stress in the tolerant *Musa* hybrid variety BRS Princesa. Leaf RNA samples were extracted following pathogen challenge, following drought stress, following simultaneous co-stresses (biotic and abiotic) and in non-stressed control plants. Purified total RNA was paired-end sequenced using lllumina HiSeq 4000 technology. Following mapping of transcriptome data against the *M. acuminata* DH Pahang reference genome, from over 25000 genes, a total of 2397 differentially expressed genes (DEGs) were identified in treatments compared to controls. Of these, a total of 933 were identified following abiotic stress, 901 following biotic stress, and 563 following the co-stress. Examining all DEGs, 38 were common to all the treatments. To understand how DEGs were involved in plant physiological responses, gene ontology enrichment analysis revealed, amongst others, repression of photoreceptors during abiotic stress and repression in trehalose biosynthesis in the biotic and co-stress treatments. Ongoing analysis of the stress-responsive molecules through KEGG and Mapman metabolic pathway analyses will further elucidate stress tolerance mechanisms, with DEGs potentially appropriate for introgression in *Musa* breeding programs.

A2.R16 - Mathematical models to explain the influence of 3D bioprinting parameters on final constructs

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Three-dimensional (3D) bioprinting is a biofabrication technique derived from additive manufacturing that consists in controlled deposition of biomaterials aiming structures production. To bioprint, parameters are set in the

equipment software so the process can be fully automated. The combinations among these parameters can be made in a non-systematic way based on user experience, following gradual optimizations according to the desired result, or through adjustments of mathematical models that can be used to make predictions of expected results. Speculatively, by using the Surface Response Methodology (SRM), mathematical models could be generated and statistically validated, a rationalized approach to optimize parameters settings. These models would allow understanding the significance of different variables involved in bioprinting process. In this study, by using the Central Composite Design, an SRM type, it was possible to propose different conditions under which it would be possible to perform experiments aiming to adjust to a reliable mathematical model. In the Chemoface software (Design type > Central Composite), three parameters (variables) and the maximum and minimum values they could assume were defined, being these, needle diameter (ND): 17.62 and 22.38 G; extrusion multiplier (EM): 0.0058 and 0.02014; printing speed (PS): 2.823 and 8.177 mm/s. From these variables values, 15 different experimental conditions were presented. Experiments were performed using a 3D bioprinter under development (prototype version) and the results considered for the model adjustment were related to the percentage of the difference between the width of the bioprinted filament (average of the measure of 30 points) and the diameter of the needle in relation to the diameter of the needle. A linear mathematical model was adjusted and statically validated, and ND and EM parameters were significant in relation to the value (percentage of difference) considered to the model.

A2.R17 - Mathematical models to explain the influence of 3D bioprinting parameters on final constructs

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Lignocellulosic biomass biorefineries aggregate value to plant biomass, converts its components (cellulose, hemicellulose, and lignin) into products, such as bioenergy, utilizing a combination of technologies and processes. Microorganisms hydrolyze lignocellulosic substrate into fermentable sugars. Naturally, the complete degradation of lignocellulosic biomass is achieved by the synergistic action of several organisms, each responsible for degrading different portions of this plant biomass. Hence, fungi co-culture is a solution to enzymatic cocktails' high prices. *Trichoderma reesei* RUT-C30 (C30) and *Aspergillus niger* (AN) are efficient producers of hydrolytic enzymes widely used in industry. This work aims to characterize the secretome and protein complexes produced in submerged co-cultures of *T. reesei* RUT-C30 and *A. niger* in different carbon sources. A previous study showed that co-cultivation could increase the enzymatic activity when *T. reesei* was inoculated first in the medium (ALVES et. al., 2020). Here, both fungs were cultivated in mono and co-cultures (carbon source sugarcane bagasse), then inoculated in supplemented mediums (AN, C30, AN+C30, AN+C3024h, AN+C3048h, C30+AN24h, C30+AN48h) agitated (120 rpm at 28°C) for nine days. Aliquotes of all samples are collected each day to construct the enzymatic curve and cellulase, xylanase, pectinase, and mannanase activity analysis. Electrophoresis (SDS-PAGE and BN-PAGE) and high-performance liquid chromatography-mass spectrometry (LC-MS/MS) of the secretomes to an inspection of multienzyme complexes will be performed.

A2.R18 - Quantification of Error Sources Accounting for Misidentification of Protein Partners in Coevolutionary Approaches

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Physical interactions across amino-acid contacts between proteins are maintained along evolution by means of compensatory mutations. Despite recent advances in the field, mostly rooted on mutual information (MI) correlation analysis, the predictive problem of protein partners remains unsolved for sequence ensembles in general, especially because an effective non-degenerate heuristic to search for the correct set of protein partners across the space of potential matches still misses in case of a large number of sequences. Scrambling simulations of the native arrangement of two proteins A and B were recently shown to generate random models with a minimum mutual information content within a few iterations steps. On the other hand, simulations that start from the very same random arrangements were found to fail at pairing native sequences correctly, with errors arising from mismatches among (i) similar and (ii) non-similar sequences after maximization of MI. We contribute here with a statistical framework to describe the probability distribution of interaction models of proteins A and B for a large number of sequences that feature a unique "native" arrangement at a maximum MI content. We show that the distribution of mutual information across the entire space of possible matches may be conveniently dissected according to the TP rate in which $0 \leq n \leq M$ sequences A and B are identically paired to their native arrangement. According to that partition, the MI probability density is described as a n-component Poisson mixture of log-normal distributions with a well-defined set of expectation values and variances. Thus, this model can tell us about the propensity of a protein system to generate errors between (i) similar and (ii) non-similar sequences.

A2.R19 - Proteomic and phosphoproteomic analysis of *Trypanosoma cruzi* interaction with macrophages

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The proposed project aims to contribute to the various researches related to the host-parasite interaction during the innate immune response, however, for the first time, considering the macrophage-*Trypanosoma cruzi* interaction, a broad proteomic and phosphoproteomic analysis of the macrophage in response to this will be carried out. pathogen. The complex molecular change of the macrophage in response to a particular pathogen, in temporal performance, can be measured in a limited number of clusters within categories, each with its own kinetic parameters detecting regulatory options and homeostatic programs. The aim of the project is to determine the molecular changes, in proteomic and phosphoproteomic terms, that occur in macrophages after infection with trypomastigote forms of *T. cruzi* and, alternatively, comparative analyzes with the protozoan *Phytomonas serpens*. Methodology: Standardization of THP-1 macrophage culture (ATCC-TIB-202), and interaction assays with parasites. Comparative proteomic analysis of macrophages infected with trypomastigote forms of *T. cruzi* and specific controls. Comparative phosphoproteomic analysis of macrophages infected with trypomastigote forms of *T. cruzi* and specific controls. Results: Note: The morphology and quantification of THP1 cells (ATCC-TIB-202), after stimulation with PMA (50ng/mL), were not satisfactory to continue with the proteomic analysis. Then, I modified the amount of PMA as well as the incubation time with PMA (100nM/ml). Standardization of THP-1 macrophage culture (ATCC-TIB-202), and interaction assays with parasites: 1-Storage preparation: THP-1 cells; blood trypomastigotes of *T. cruzi,* promastigotes of *P. serpens*; 2-Tests regarding the concentration of phorbol to be used; 3- Number of cells and concentration of phorbol in the culture; 4-Number of parasites/cell; 5- Culture incubation time.

A2.R20 - Characterization and synergistic analysis of lignocellulosic biomass bioconverting enzymes, bioprospected from termite intestine metagenome *Syntermes Wheeleri*

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Microorganisms have been used as an important source for bioprospecting, and they correspond to the world's second largest biomass in Gigatonnes of carbon, just behind plants. Metagenomics allows accessing the universe

of microbes that are not-cultivable in traditional laboratory environments, for various applications, whether for ecological or biotechnological purposes. In this work, metagenomics was used as a tool for bioprospecting cellulase enzymes (endoglucanases, cellobiohydrolases and β-glucosidases), resulting from the microbiota of the termite gut, *Synthermes wheeleri*, an abundant species in the Brazilian Savanna, Cerrado. These insects are promising in the decomposition of lignocellulose and are responsible for consuming worldwide, something around 3 to 7 billion tons per year. At this work, primarily was done a selection of potential sequences from the library, followed by a screening using the metatranscriptome results, and so synthesized and expressed in Escherichia coli BL21 (DE3). Phylogenetic analyzes indicated Firmicutes, Proteobacteria, Bacteroidetes and Spirochaeta as the most abundant phyla among the selected sequences. As for enzymatic analyses, the conserved domains (carbohydrate-binding modules) for Glycosyl Hydrolases (GH) were noted as GHs 3, 5, 9 and 10. In addition the presence of active site were checked to ensure the activity of the enzymes. At the end, three proteins were chosen for biochemical and biophysical characterization, in order to perform the synergism between them in different biomasses, and see their potential as an application product. The proteins were confirmed by SDS-PAGE, and optimized for better yields of concentration. Mostly present in inclusion bodies, the use of solubilization techniques was of extreme significance, allowing purification steps. The analysis of biomass degradation products' will be done by X-ray fluorescence (XRF), and the surface decomposition by Scanning Electron Microscopy (SEM).

A2.R21 - Analysis of the impact of anesthetics binding on the balance between states of the Kv1.2 channel directly from flooding simulations at high concentrations

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General anesthetics are drugs that are fundamental to medicine, but despite their use for over two centuries, the molecular mechanism of general anesthesia remains unknown. The difficulty to clarify this issue is due to the fact that they produce similar effects, but do not have a similar structure, and studies have shown that channels have different sensitivities to anesthetics. One of the theories that have emerged to explain this phenomenon is the disturbance of the balance between protein states due to anesthetic binding, where the probability of occurrence of each conformation depends on the anesthetic binding energy with each state. To investigate this conformational disorder, the voltage-dependent Kv1.2 channel was chosen because it has characterized open and closed conformations and electrophysiology studies have shown different responses to sevoflurane, which activates the channel, and propofol, which does not alter its activity, thus offering a negative control for analysis. Flooding simulations at high concentrations, a computational method where the channel is balanced in the presence of an environment saturated with ligands which bind to the protein over multiple degenerate regions at reversibly and finite rates, were performed to obtain the spatial probability density ρ(R) of both anesthetics for the Kv1.2 channel in open and close states. The binding probabilities were then compared with curves obtained through a toy model based on the theoretical framework. The partition functions for both states were then calculated from the model and used to estimate the voltage-dependent open probability po(V) of Kv1.2 in the presence of these anesthetics. The simulations were able to show that sevoflurane has a higher affinity for the open conformation whereas propofol has very similar affinity for both states. Thus, the model, in agreement with experimental data, exhibits a large negative shift of po(V) for sevoflurane and a slight positive shift for propofol.

A2.R22 - Structural and functional studies of C24-methyltransferase sterol enzymes from human pathogenic fungi *Candida auris* and *Aspergillus fumigatus* aiming at the development of potential antifungal drugs

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Invasive fungal infections are a major cause of mortality in immunocompromised patients caused most commonly by *Candida* and *Aspergillus* species. *Candida auris* species stand out with high mortality rates. Ergosterol is an essential lipid for the cell viability by being responsible for membrane fluidity and permeability. Cholesterol and ergosterol share the same metabolic pathway until the stage of zymosterol synthesis, from this point onwards they diverge and use different enzymes. The gene erg6 encodes a 24-C-methyltransferase (SMT) that acts transferring C-24 methyl group converting zymosterol into fecosterol or acts in an alternative pathway converting lanosterol to eburicol. The SMT is a promising target because it is present in invasive fungi and absent in the human host. The soluble CauSMT (C. auris) and AfSMT (A. fumigatus) was purified by immobilized metal affinity chromatography. The effect of additives on the oligomeric state of the proteins was analyzed by size-exclusion chromatography and dynamic light scattering which showed a pentameric CauSMT and a tetrameric AfSMT in a specific condition. Thermal stability as a function of pH was analyzed by circular dichroism. This analysis demonstrated that CauSMT and AfSMT were more stable at an acid and neutral pH. The melting temperature of both enzymes at the most stable pH was 59 °C. Enzymatic assay showed that CauSMT has specificity for zymosterol and AfSMT uses the alternative pathway converting lanosterol to eburicol. The structural models of both enzymes were predicted by homology modeling and deep learning. They will be used for the studies of substrate/ligand-interaction specificity. In pursuit of the development of potent and selective drugs, crystallographic, enzymatic and inhibition studies are being carried out.

A2.R23 - Reconstruction of Dilute Binding Affinities of Small Ligands from High Concentration Molecular Simulations

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A wide range of small molecules interact with proteins, leading to several physiological changes, which some are the mechanism behind pharmaceutical procedures. Some proteins offer ligand-specific binding sites, characterizing a high-affinity interaction. However, it has been shown that this is not the only ligand-protein interaction mechanism: some small molecules can bind throughout the protein surface, with low-affinity interactions, causing a huge net cooperative affinity. The latter type of interaction has been very challenging to characterize, because few techniques are able to handle these small affinity interactions. An example of small ligand modulation is the interaction between the voltage-gated potassium channel Kv1.2 and the general anesthetic sevoflurane. Sevoflurane is known to activate Kv1.2 channel at 1 mmol/L concentration, but its mechanism is still unknown. As stated before, the interaction is very complex, with many degenerate states due to the big protein size, so to seek faithful descriptions of Kv1.2-sevoflurane behavior, we utilized flooding-MD simulations, which is a computational method that reproduces their interactions in atomic scale at a fixed ligand concentration. However, to achieve informations at dilute concentrations, it would require enormous simulation time to achieve convergence. To solve this problem, we performed high concentration MD simulations (25, 50, 75, 100 and 150mM) to enhance sampling and developed a theoretical framework to retrieve information about the low concentration state (1mM control simulation). The framework describes the sevoflurane interaction using an ideal

thermodynamic cycle, and each step of this cycle has a contribution to the calculation of the binding affinity. The theoretical model is also capable of dealing with the non-ideality of the components and reproduces successfully the interactions in lower concentrations, offering a good tool to study these complex and cooperative interactions.

A2.R24 - Evaluation of serine-integrases activity as genome editing tools in the synthetic minimal cell *Mycoplasma mycoides* JCVI-syn3B

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The identification of the set of essential and quasi-essential genes for the assembly of the minimal cell approximation *Mycoplasma mycoides* JCVI-Syn3B put us closer to understanding the basic molecular requirements to life. The transposon bombardment methodology originally used allows the screening of every gene in the cell through transposon insertion and gene disruption, although only one loss-of-function event can be observed in a single cell. However, genetic redundancy and functional complementarity make it of interest to evaluate gene essentiality in a broader context of gene clusters. We propose that serine-integrases acting on defined groups of genes can work as such systems. We first evaluated the use of two serine-integrases (INT9 and INT13) as genetic switches capable of turning the expression of a reporter gene on by flipping its DNA sequence upon induction in the synthetic minimal cell *Mycoplasma mycoides* JCVI-Syn3B. Cells were transformed with a plasmid containing one integrase gene under control of a Tetracycline inducible promoter as well as the mCherry gene in a reverse orientation relative to its promoter, therefore silenced, flanked by the att sites of that integrase. All the plasmids also had a Cre/loxP system to allow insertion on a landing pad present in the Syn-3B genome and a selection marker. Following incubation with tetracycline, we measured mCherry signal intensity in a microplate reader. Results show that INT9 activation led to a significant increase in fluorescence intensity, with no observed signal in non-induced groups, and the expression of the flipped reporter gene continued after tetracycline removal. INT13 induction did not result in apparent expression of mCherry, although reporter inhibition in one of the positive controls suggests an influence of the sites rather than INT13 ineffectiveness. Preliminary results obtained with INT2 also indicate no recombinase activity upon induction, but further investigation is necessary, especially regarding vectors design. Next steps include insertion of INT9 att sites flanking multiple endogenous genes of syn-3B and improving of INT2 and INT13 systems.

3. Genética, genômica e evolução (A3)

A3.R1 - Ship: Annotation-based program for identifying Genomic Safe Harbours in eukaryotic model organisms

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Since the beginning of genetic engineering, the insertion of exogenous genes into host cells has presented itself as an important challenge. With time and the advent of Synthetic Biology, the challenge became even greater with the expansion of monogenic to polygenic traits of interest and the need to insert metabolic pathways and entire genetic circuits. Targeting inserts to genomic regions allowing their expression without altering endogenous gene expression is a desirable design approach for the integration of exogenous genes into host cells. Such regions, classified as Genomic Safe Harbours (GSHs), have been identified by viral insertion site, analysis of loss of gene function, or similarity to GSHs from other organisms. Available GSHs are predominantly located in intragenic loci, with high genetic density and close to oncogenes, raising concerns about unstable expression and unpredictable or undesirable phenotypes. Given the ongoing analytical expansion, the necessity of tools for systematic and optimized identification of GSHs became clear. To expand the potential GSHs (pGSHs) available in eukaryotic model organisms, the present work developed SHIP, a computer program to identify pGSHs in intergenic regions. Here, the genomes of three model organisms were analyzed and after defining the parameters, the program identified 6 pGSHs in *Saccharomyces cerevisiae*, 11 in *Mus musculus*, and 16 in *Homo sapiens*. Due to the high level of genomic annotation, the latter organism was chosen to be combined with machine learning to improve the pGSH analysis. This model achieved 97% accuracy in the training phase, was able to identify all classes in the test samples and when used with the pGSHs predicted by SHIP, all pGSHs were classified as containing coding sequences. SHIP predicts GSHs and is a general-purpose tool to provide an investigative start by reducing the pGSHs that need to be investigated with other bioinformatics resources and validated with experimental data.

A3.R2 - Scalable and versatile pipelines for comprehensive bacterial genomics analyses

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Advances in DNA sequencing technologies are reshaping bacterial genomics studies by enabling chromosome level assemblies, at a fraction of cost and time, paving the way to population level genomic surveys. Currently, the computational analysis of sequencing data is the main hindrance to the field and withholds its move into mainstream clinical settings. To overcome this barrier we developed a comprehensive set of container-based pipelines, written in Nextflow, designed to assist users in bacterial genomics analyses providing common workflows with straightforward execution for inexperienced users, but also with high levels of customization for the most experienced ones. Divided in three modules, the pipelines perform the tasks of quality control, genome assembly and annotation. After initial raw data processing by the quality control step, genome assembly is performed by a variety of programs and strategies depending on the DNA sequencing technology employed. The genome annotation pipeline was designed to provide a generic but comprehensive overview of the genome, performing tasks such as virulence and antibiotic resistance genes annotation, methylation calling, prediction of prophages, genomic islands and plasmids. The annotation results are summarized in reports and also can be visualized in integrated genome browsers. Finally, a web-based interface is also available to provide a quick way to explore and interact with the genome annotation results. The pipelines and the web-based interface are very plastic, meaning that modules can be rapidly added to match the community needs. The modularity and scalability of the pipelines makes them suitable for any project size, from one to thousand genomes. In conclusion, these ready-to-use pipelines offer a seamless exposition of computational tools to help in genomics studies while also bridging the gap toward routine clinical bacterial genomics.

A3.R3 - sRNA-Seq analysis of *Arachis duranensis* (peanut) root samples subjected to cross stress conditions (drought and root-knot nematode inoculation)

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Population growth and climate change are important challenges faced by humankind. The main stress components predicted to influence crop growth and development are drought stress and root-knot nematode (RKN) parasitism. The discovery and deeper understanding of post-transcriptional regulatory mechanisms, combined with the development of high-throughput sequencing technologies and computational tools for data analysis, have enabled considerable advances in research into microRNA molecules and their roles in gene expression modulation. Through sRNA-Seq analysis, the present work aims to characterize miRNA diversity and differential expression in root tissue samples in *Arachis duranensis* plants (peanut), a well-suited model, inoculated with *Meloidogyne arenaria* (a root knot nematode species) (INOC), and inoculated with *M. arenaria* and simultaneously exposed to drought stress (STR), all in comparison with unstressed control plants. A total of nine cDNA libraries (three biological replicates for each treatment, including control samples) were constructed and sequenced using Illumina technology, with an average of 49M, 46M and 37.6M sequences generated per library for the control, INOC and STR, respectively. Raw data was pre-processed using Cutadapt software for adaptor removal and sequence size selection (20-24nt), followed by contaminant removal using Bowtie. Subsequently, a total of 18.1M, 15.7M and 8.4M reads for control, INOC and STR treatments, respectively, were selected for further analysis in the next steps of the pipeline. Alignment and prediction of miRNA families was performed with the software packages ShortStack v. 3.4 and Mireap v. 0.2. Annotation of miRNA families was performed using Blastn with miRbase v. 22. StrucVis0.3 software was used to predict the hairpin secondary structure. As preliminary results, 143 putative miRNAs from 29 miR families were identified. The next steps include miRNA differential expression analysis and miRNA target prediction

A3.R4 - Study of gene knockout by CRISPR of a candidate water tolerance gene in the genome of *Solanum licopersicum* (Micro-tom)

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Data subject to intellectual protection

A3.R5 - A novel strain of *Klebsiella pneumoniae* isolated from an urban lake possesses multidrug resistance and a novel carbapenemase transposon

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Antimicrobial Resistance (AMR) is an increasing issue worldwide and its dissemination within and outside of hospital contexts is of vital importance to diminish AMR costs on human morbidity and mortality. In virtue of the COVID-19 pandemic, we decided to analyse the genome of a bacterial strain isolated from Paranoá lake, originally collected for the biodegradation of organic xenobiotics, as to identify genes related to its virulence and AMR. The strain was identified as belonging to the *Klebsiella* genus by 16S sequencing and it presented a multiresistant

phenotype by the disk diffusion assay. Genomic analysis identified the isolate as *K.pneumoniae*, albeit in silico Multilocus sequencing tagging shows it belongs to a novel Strain Type: ST5236. ANI-based phylogeny with genomes from the National Center for Biotechnological Information (NCBI) further support this isolate as representative of a novel strain. Resistome analysis indicates the presence of diverse AMR genes, such as efflux pumps, beta-lactamases and genes for heavy metal resistance. Notoriously, a blaCTX-M-15 gene, which is related to the resistance of most beta-lactam antibiotics, was found in close proximity to a Siphoviridae prophage sequence and a KPC-2 gene, which is related to resistance against last-resort beta-lactams, was found in an extrachromossomal contig and associated to an undescribed transposon. Moreover we have found nearly complete gene clusters of the type VI secretion system and three VgrG4-like effector genes, which may contribute towards competition interactions with fungi and other bacteria. One contig of this isolate was also nearly identical (>99%) to contigs from a hospital *K.pneumoniae* strain, which points to a potential gene flow from hospitals in Brasília into the Paranoá lake. The discovery of a gene flow is concerning, as the city has undergone periods of water scarcity and thus turned the lake into a source of drinking water reuse and is also used for recreational purposes.

A3.R6 - Obtainment of soybean cultivar resistant from whitefly with RNA interference strategy

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The whitefly (*Bemisia tabaci*), is one of the major agricultural pests in several parts in the world, it causes damage due to its feeding on the phloem and it provoke alterations in the vegetative and reproductive development of plants. The big capacity to reproduce and adapt to adverse conditions, makes the whitefly quickly become resistant to many insecticides. Given the importance of soy as a source of protein and vegetable oil and the difficulties in controlling whitefly in crops, it is essential to find sustainable alternatives for its control. Genetic engineering techniques can be used in the development of resistant plants, such as gene silencing through RNA interference (RNAi). Therefore, the objective of this research is the development of soybean lines resistant from whitefly using the RNAi strategy. The development of plasmid vectors is an essential tool in the generation of a transgenic plant, as it allows the transport and manipulation of exogenous DNA within a host cell, so the first step executed of this research was the construction of a transformation vector looking for the expression of dsRNA corresponding to a gene sequence of a whitefly vATPase. The dsRNA expression cassette contains a fragment of the gene of interest under the control of the 35S promoter (Cauliflower mosaic virus - 35SCaM); the Atahas gene that confers resistance to herbicides of the imidazolinone class and the neo gene (nptII) that confers resistance to kanamycin. The vector developed is being used for soybean transformation by the bioballistic system, it is expected that the siRNAs are expressed by the transformed plants and can be absorved by *B. tabaci* and leads to the silencing of vital genes.

A3.R7 - Contrasting transcriptomic profiles in resistant and susceptible cupuassu (*Theobroma grandiflorum*) challenged with *Moniliophthora perniciosa*, the causal agent of witches' broom disease

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Theobroma grandiflorum is a fruit tree native to the Amazon region and a relative of cacao (*T. cacao*). It has a huge economic potential due to its multiple uses in the food and cosmetic industries. Most of the commercial varieties are susceptible to *Moniliophthora perniciosa*, a fungus that causes witches' broom disease (WBD) in both cupuassu and cacao. Knowledge about this phytopathosystem is essential for proposing strategies to control and mitigate the damage caused by WBD to these cultures. To provide insights into cupuassu resistance to *M. perniciosa*, the transcriptomic profiles of a resistant (clone 174) and a susceptible genotype (clone 1074) were analyzed using RNA-seq technology. In this study, we present the analyses of the transcriptome of both genotypes challenged with *M. perniciosa*, in the early stage of infection. A total of 21,441 unigenes and 440 differentially expressed genes (DEGs) were identified among the different conditions. The intrinsic difference analysis between the genotypes showed 301 DEGs. Gene expression alteration was observed earlier in the susceptible genotype at 24 hours after inoculation (HAI). In the resistant one, the alteration was more prominent at 48 HAI. These data set allowed the identification of genes potentially involved in the mechanism of defense, among them, pattern-recognition receptors (PRRs), transcription factors, pathogenesis related proteins (PRs), proteins related to cell wall remodelling, genes related to reactive oxygen species (ROS) accumulation and terpene pathways. The phytohormone signature analysis revealed a significant hormonal influence in genotypes' responses. The genotype differed mainly relatively to auxin, cytokinin, salicylic acid and brassinosteroids responses. This is the first large-scale transcriptome study of *T. grandiflorum*. Our results revealed critical molecular differences between contrasting cupuassu genotypes with respect to resistance to WBD.

A3.R8 - Chromosome-level assembly of the industrially relevant fungus *Aspergillus terreus* ATCC20542 using nanopore sequencing

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Secondary metabolites (SM) found in filamentous fungi are either unique or distributed among few species, and are produced by a series of chemical steps orchestrated by several genes, usually arranged in defined genomic loci, the biosynthetic gene clusters (BGCs). Comparative studies have shown within-species BGC diversity, even with the whole BGC being absent in different strains of the same species. As such, a single genome is not a representative of the potential of SMs that species may synthesize. *Aspergillus* sp. is a fungal genus that illustrates the natural abundance of SMs that can be harnessed for biotechnological and biomedical use. Particularly, *Aspergillus terreus* is known for its ability to produce a SM used in the past as a cholesterol lowering drug, lovastatin. In this study, we obtained a high quality genome sequence of *Aspergillus terreus* ATCC 20542, the prototype strain for lovastatin production, using Oxford Nanopore's MinION sequencing device in a cost and efficient manner. A de novo genome assembly generated 15 chromosome-scale contigs (N50=4.02 Mb). We compared the resulting assembly against *A. terreus* reference genome, strain NIH2624, to define the genomic characteristics that mark each strain lifestyles since the former is a soil isolate and, the latter, a clinical isolate. In functional terms, we focused on the potential for SM production. The entire BGC of terretonin was absent from *A. terreus* ATCC20542 genome. Terretonin has been detected among the metabolites associated with conidia germination processes preceding Aspergillus infections. The exclusive detection of terretonin BGC in NIH2624 corroborates the hypothesis of SMs influencing intra-species lifestyle. The genomic information obtained in this study is valuable to understand the link between the different lifestyles among same species strains.

A3.R9 - Characterization of miRNAs in *Musa acuminata* during interaction with *Pseudocerspora musae*

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Endogenous microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level by cleavage or repression of mRNA translation. Banana (*Musa* spp.), a monocotyledonous crop cultivated throughout tropical regions, is susceptible to numerous diseases. *Pseudocercospora musae*, the causal agent of Sigatoka leaf spot disease, is an important fungal pathogen of banana. Here, leaf RNA samples were extracted from *Musa acuminata* subsp. *burmannicoides*, var. Calcutta 4 (resistant), at 3 and 12 days after inoculation (DAI) with conidiospores. Following small RNA library construction, samples were sequenced using Illumina HiSeq 2500 technology. High quality sequences were mapped against the *M. acuminata* ssp. *malaccensis* var. Pahang reference genome and plant miRNAs were predicted using the programs Mireap and ShortStack. A total of 228 loci were observed that contain miRNAs belonging to 30 distinct miR-families and 22 novel miRNAs. A total of 48 known miRNAs from 25 families and two novel Musa miRNAs displayed delta values in excess of 100 sequences between 3DAI_I and 3DAI_NI treatments. The delta values of the four miRNAs most highly expressed at 3DAI_I (miR397, miR398, miR408 and miR827) varied between 167 and 768 in relation to 3DAI_NI. Delta values for 44 miRNAs down-regulated at 3DAI_I in relation to 3DAI_NI varied between -51.484 and -118, with miR160 and miR530 significant on the basis of edgeR analysis. Across 12DAI, 31 miRNAs, from 18 known families and two novel Musa miRNAs, displayed up- or down-regulation between 12DAI_I and 12DAI_NI treatments. Of these, 9 displayed up-regulation at 12DAI_I, with delta values between 230 and 8999, and 21 displayed down-regulation at 12DAI_I, with delta values between -15603 and -102. Analysis of miRNAs in *M. acuminata* and their role in gene expression modulation during interaction with *P. musae* provides resources for the development of efficient methods for control of Sigatoka leaf spot disease.

A3.R10 - Induction of defense-related genes in Cabbage using concentrated metabolites produced by *Rhizobium tropici*

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To verify the potential of metabolites extracted from *Rhizobium tropici* to trigger the priming of defense responses in cruciferous plants, we analyzed the expression of defense-related genes by qRT-PCR. *Brassica oleracea* var. *capitata*, susceptible to *Xanthomonas campestris* pv. *campestris* (Xcc), were grown in greenhouse conditions. At 18 days after sowing, plants were inoculated with 1 mL of 1% concentrated metabolites produced by *R. tropici* (CM-RT) in the root. In a second experiment, leaves were sprayed with 1 mL of a solution containing 1% CM-RT. Aerial and

root tissue were collected separately at 0 (non-treated control condition), 24, and 48 hours after treatment (hat), submitted to RNA extraction and gene expression analysis by qRT-PCR. The results showed that after root treatment with CM-RT, most evaluated genes were upregulated at 24 hat and downregulated at 48 hat in roots, while in leaves, genes were downregulated both at 24 and 48 hat. On the other hand, leaf treatment with CM-RT showed that most evaluated genes in leaves and roots were upregulated at 24 and 48 hat. These results indicate that the effect of CM-RT applied in roots seems restricted to the applied region and is not sustained, while the application in leaves results in a more systemic response and maintenance of the effect of CM-RT for a longer period. The results obtained in this study emphasize the biotechnological potential of using metabolites of *R. tropici* as an elicitor of active defense responses in plants

4. Biologia celular e molecular de microorganismos (A4)

A4.R1 - Optogenetic control of gene expression in *Komagataella phaffii*

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The yeast *Komagataella phaffii* (*Pichia pastoris*) has been used as a platform for the production of heterologous proteins for over 20 years due to advantages such as high levels of gene expression with the methanol-induced AOX1 gene promoter (PAOX1). However, methanol is a flammable chemical inducer. In recent years, optogenetics has become a widely used tool for the regulation of biological processes such as gene expression, protein localization, signal transduction and protein-protein interactions. Optogenetics is based on the use of light, a physical inducer, for the induction of adjustable systems. The application of an optogenetic circuit for the regulation of gene expression in *K. phaffii* will allow the use of an induced system that does not suffer interferences from cell metabolism. In the present project we will seek the development of an optogenetic circuit capable of regulating *K. phaffii* PAOX1 promoter activity in a methanol-independent manner. For the construction of the circuit, the PHYB and PIF3 photoreceptors of Arabidopsis thaliana, which respond to red/infra-red light, and the phycocyanobilin chromophore (necessary for PHYB activation) were chosen. We will use a double-hybrid system with the aforementioned proteins, the DNA-binding domain of the transcription factor MIT1 and the transactivation domain of VP16 protein. Initially, the EGFP gene will be used as a reporter, and a biopharmaceutical or protein of interest in the food industry will be used to validate the system. At this moment, we have all the constructions ready and transformed in the Xhis23 strain. In addition, gene expression was verified by RT-PCR. From there, tests were carried out to assess the expression of eGFP in the presence of red light.

A4.R2 - Heterogeneity in the Response of Different Subtypes of *Drosophila melanogaster* Midgut Cells to Viral Infections

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Single-cell RNA sequencing (scRNA-seq) offers the possibility to monitor both host and pathogens transcriptomes at the cellular level. Here, public scRNA-seq datasets from Drosophila melanogaster midgut cells were used to compare the differences in replication strategy and cellular responses between two fly picorna-like viruses, Thika

virus (TV) and D. melanogaster Nora virus (DMelNV). TV exhibited lower levels of viral RNA accumulation but infected a higher number of enteroendocrine cells compared to DMelNV. In both cases, viral RNA accumulation varied according to cell subtype. The cellular heat shock response to TV and DMelNV infection was cell-subtype- and virus-specific. Disruption of bottleneck genes at later stages of infection in the systemic response, as well as of translation-related genes in the cellular response to DMelNV in two cell subtypes, may affect the virus replication.

A4.R3 - RNAi-Mediated Parasitism Gene Silencing: A Novel Source of Resistance for Crop Protection against the *Meloidogyne incognita*

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Plant-parasitic nematodes are widely distributed and pose a serious threat to crop production worldwide. Several practices, including chemical, biological, and tolerant cultivars have been frequently applied for the management of the root-knot nematode (RKN), *Meloidogyne* spp. However, due to the non-specificity of these approaches, the associated risks to human health and the environment, and the potential development of resistance in tolerant cultivars, new biotechnological strategies are demanded. The use of RNA interference (RNAi), a post-transcriptional gene silencing process, has shown to be a potentially promising approach for the control of RKN. In this study, RNAi-mediated gene silencing of the Minc03328 and Minc16803 parasitism genes were applied to the generation of transgenic *Arabidopsis* plants. For the dsRNA expression vector, we designed a T-DNA Construct with full-length uceS8.3 regulatory region of the soybean E2 ubiquitin-conjugation gene (GmUBC4), a nematode-responsive promoter, modulating the hairpin-type dsRNA (nuHP-dsRNA) expression in the nuclear genome of *Arabidopsis thaliana*. Data demonstrated that transgenic *Arabidopsis* plants expressing the nuHP-dsRNA targeting Minc03328 and Minc16803 transcripts demonstrate significant increased resistance to *Meloidogyne incognita* infection. Gall numbers and egg masses were reduced by up to 81% and 93%, respectively, in the At-nuHP-Minc03328 transgenic lines, whereas nuHP-dsRNA targeting Minc16803 showed 76% and 87% reduction for At-nuHP-Minc16803. Interestingly, histopathological analyses of M. incognita-induced galls strongly suggest that Minc03328 and Minc16803 gene may play an important role during early parasitism stages, encompassing amorphous giant cells with lower cytoplasm content in transgenic lines, besides a hallmarked effect over nematode cuticle, reinforcing its potential as a promising specific target for application in modern crop protection development.

A4.R4 - In silico description of uncultured bacteria from a sponge microbiome

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The ocean is a rich environment composed of distinct species from the three domains of life that can be explored in different ways. Among marine organisms, there is a special interest in sponges due to their capacity to harbor highly diverse microorganisms and to produce secondary compounds. This project concerns the in silico description of 10 uncultured bacteria's genomes recovered from the metagenome of a sponge located in the Amazon Reef,

Brazil. They were initially classified in the candidate phylum Handelsmanbacteria. The main focus is to determine the ecological and biotechnological relevance of these bacteria through genomics analysis. Genomes' qualities were determined with the software CheckM. Metabolic reconstruction was made using Kegg and Metacyc databases. Relevant enzymes were found using the BRENDA database while secondary metabolites were searched with antiSMASH web server. All genomes have completeness greater than 70% and contamination smaller than 10%. The bacteria analyzed have an energy metabolism similar to heterotrophs, including central carbon metabolism and oxidative phosphorylation. Some of them can also do fermentation and anaerobic respiration using sulfate. The metabolic reconstruction showed that these bacteria not only contribute to the community through bioremediation of heavy metals and harmful organic compounds, and participation in biogeochemical cycles like carbon, nitrogen, phosphorus, and sulfur; but can also benefit the sponge by providing nutrients and helping in the host protection from hazardous substances, pathogens and predators. From a biotechnological perspective, these bacteria participate in the production of many enzymes, antibiotics and other secondary metabolites that can be applied commercially, industrially and medically.

A4.R5 - Elucidation of the primary structure of the TfTx peptide, isolation of the scorpion *Tityus fasciolatus* and evaluation of activity in ion channels Kv 1.1, Kv 1.2 e Kv 1.4

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Ion channels are transmembrane proteins that selectively allow the passage of ions through them, moved by the existing electrochemical gradient. These proteins are classified according to ion selectivity and mechanism of action, being involved in several physiological activities, including nervous impulse transmission (Beirão, 2009; Júnior, 2009). Among the different channels are the K+, channels, responsible for maintaining cell excitability. This group houses a great diversity of channels, divided into four families: the voltage-dependent channels (Kv), Inwardly Rectifying Potassium (Kir), Two-Pore Potassium Channel (K2P) and Calcium-activated Potassium Channel (KCa). The Kv and Kca are the main targets of scorpion toxins between potassium channels, acting mainly on the Kv1 channel subfamily, which are abundantly present in nervous tissues (Choe., 2002, Gati et al., 2012, Morales Duque., 2013). The TfTx, a peptide isolated from the venom of *Tityus fasciolatus*, has a molecular mass of 3.583,64 Da [M+H]+ and its partial sequence revealed 19 amino acid residues with the presence of 3 disulfide bridges. In comparisons made in databases, it was observed that this peptide has a high degree of similarity (78%) with 2 other peptides: CllNtx isolated from the venom of *Centruroides limpidus* and 'Peptide A' isolated from *Centruroides hirsutipalpus* (Valdez-Velázquez et al.,2018). This group of peptides has no physiological activity described and studies carried out with these molecules suggest that they compose an not yet described family of neurotoxins with possible activity in potassium channels (Uribe et al., 2017; Valdez-Velázquez et al.,2018).

A4.R6 - Adaptive immune receptor repertoire sequencing of SARS-CoV-2 vaccinated individuals

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The adaptive immune receptor repertoire sequencing (AIRR-Seq) consists in a major approach for studying celular and humoral adaptive immune responses against a wide variety of antigenic immune challenge. By employing high throughput sequencing tecnologies, it became possible to obtain massive and highly precise amount of data that

allows to gain insight into the characteristics, dynamics and properties of repertoires of T and B cell receptors (BCR). The SARS-CoV-2 represents a major world health issue, having infected and killed millions so far. Several vaccines have been developed since the beginning of the pandemic, and have been crucial to control the virus spread. In Brazil, CoronaVac, a virus inactivated like-particle vaccine, was massively used by the Brazillian national immunization program. Whatsoever, aspects about how CoronaVac induces immunity and how it shapes immune humoral response are yet to be better understand. Thus, the objective of this work is to analyze BCR repertoire sequencing data of individuals vaccinated against SARS-CoV-2. Peripheral blood samples of vaccinated individuals are still being collected. Once it's finished, samples will be sequenced. The raw BCR sequencing data will be analyzed by immcantation, a bioinformatics frawework specialized for repertoire data analysis. It's intented to understand more about the repertoire of vaccinated individuals and, possibly, identify signatures of repertoire convergence between samples that might allow the isolation of broadly neutralizing antibodies against SARS-CoV-2.

A4.R7 - Occurrence of mixed infection of lettuce chlorosis virus and cowpea aphid-borne mosaic virus in *Passiflora* spp. in Brazil

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The Germplasm bank "Flor da Paixão"-BAG-FP maintained at Embrapa Cerrados (Brasilia, DF) harbors *Passiflora* spp. aiming at the conservation, characterization, and diversified use of Passiflora genetic resources. In 2017 diverse virus-like symptoms were observed in the *Passiflora* spp. plants from the BAG-FP. The present work aimed to investigate the viral diversity in *Passiflora* spp. from BAG-FP. Leaves of 21 *Passiflora* spp. were collected, and dsRNA was extracted, pooled, and sequenced by Illumina HiSeq2500. Bioinformatics analysis revealed contigs with identities to RNA1 and RNA2 of lettuce chlorosis virus (LCV, Crinivirus, Closteroviridae) and cowpea aphid-borne mosaic virus (CABMV, Potyvirus, Potyviridae). To verify the presence of LCV in individual samples, primer-pairs that amplify the partial RNA1-p8/p23 genes and the partial RNA2-HSP70h/p6.4/p60 were used in RT-PCR. Two of the 21 BAG-FP samples (P. auriculata, P. alata) were positive for LCV. The Sanger sequencing of the amplicons confirmed their identity of RNA1 and RNA2 as LCV (MN564795-98). The occurrence of LCV was also investigated in the samples collected in commercial fields. LCV was detected in 01/11 P. edulis from Dom Basílio (Bahia), and in 03/05 P. edulis from Planaltina (Distrito Federal). For CABMV, RT-PCR was done only in LCV positive samples with primers that amplify the partial HC-Pro/p3 genes. The mixed infection of LCV and CABMV was confirmed in all plants from Dom Basílio and BAG-FP. For the LCV-infected plants from Planaltina, two were also infected by CABMV. All CABMV RT-PCR amplicons had their identity confirmed by sequencing. CABMV is the main virus that affects passion fruit in Brazil. LCV has been reported to infect several crops. This is the first report of the natural occurrence of LCV in *Passiflora* spp. and the first report of CABMV infection in *P. auriculata*. Additional studies will be necessary to evaluate the epidemiology/impact of LCV in passion fruit.

A4.R8 - Extracellular vesicles secreted by *Trichoderma harzianum* during the interaction with their host

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Filamentous fungi of the genus *Trichoderma* are soil fungi, which can be saprophytes, mycoparasites or plant symbionts. The association between Trichoderma species and plants is beneficial to the hosts, among the positive effects we can mention: growth promotion, increase in nutrient uptake and induction of resistance to biotic and abiotic stresses. Due to its ability to parasitize phytopathogenic soil fungi, mycoparasitic activity, to induce defense response, promote growth and resistance to biotic and abiotic stresses in host plants, Trichoderma species in particular *T. asperellum* and *T. harzianum* are studied for use as biocontrol agents, biofertilizers and biofortifiers. In fact, the possibility of its use as a biocontrol agent has boosted research related to the development of commercial products based on them, as well as to the understanding of their biology and mechanism of interaction with their hosts (fungi and host plants). Commercial products based on Trichoderma, mostly *T. harzianum*, are already used in the biocontrol of *Sclerotinia sclerotiorum*, *Fusarium oxysporum*. These products represent a sustainable alternative for polluting chemical treatments, widely used in the control of fungal diseases in crops in Brazil and worldwide. Despite the availability of these products, their efficiency as a biocontrol agent varies depending on the species of phytopathogenic fungus to be controlled, from adaptation to biotic and abiotic stresses to which the biocontrol agent will be submitted. In addition to the possibility of application, *T. harzianum* is also an excellent model for studies of extracellular vesicles during the interaction microorganism microorganism and symbiotic microorganism host plant.

A4.R9 - Analysis of the role of prohibitin protein of *Aedes aegypti* in the infectious cycle of Mayaro virus

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Mayaro virus (MAYV) is an arthropod borne virus (arbovirus) of increasing medical and epidemiological relevance in Brazil and is the causative agent of Mayaro fever. The disease is characterized as an acute febrile illness in humans, but most notably, it is often accompanied by severe and sometimes debilitating joint pain (arthralgia) that can become chronic and persist for weeks or months after initial infection. Recent identification of cases in areas in Brazil where the virus was previously not usually detected increases the concern of the risk of larger outbreaks, and of the risk of urbanization of the disease mediated by the transmission by abundant urban mosquito vectors. There are currently no licensed vaccines available. Prohibitin proteins (PHB) are a conserved family of proteins with diverse function, that are involved in transcriptional regulation and mitochondrial function. Prohibitin has been identified as being a possible membrane receptor for Chikungunya virus and Dengue virus, and its expression may be regulated by viral factors during MAYV infection. In this work, we aim to investigate the possible role of PHB of Aedes aegypti cell line Aag2 as receptor for MAYV entry, as well as to identify possible viral factors that may be involved in the regulation of its expression in this cell lineage during infection. We propose using interfering RNAs to silence PHB expression, as well as blockage of PHB by monoclonal antibodies, followed by in vitro infection by MAYV to evaluate the impact on infection progression. We also aim to evaluate if the hyperexpression of specific viral genes during infection can enhance the infection kinetics, measured by the quantification of virus production by RT-qPCR. The investigation of the role of PHB in MAYV infection in Aag2 cells, and of viral regulatory factors, may be important for the further elucidation of virus-host relations and the identification of therapeutic targets.

A4.R10 - Preliminary analyses of *Chiococca alba* (L.) Hitch plant extract antiviral potential against Chikungunya and Mayaro viruses

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Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are arboviruses diseases with a high impact on public health in many countries of the neotropical regions, due to frequent disease outbreaks and epidemics. They belong to the Togaviridae family and the Alphavirus genus, and clinically, the diseases caused by CHIKV and MAYV are characterized by fever, headache, and arthralgia. Alphavirus non-structural proteins are involved in viral replication and transcription and, therefore, are potential targets for the development of inhibitors, mainly nsP2 due to their multi-action in viral replication. The use of plant-based compounds that affect the infection cycles of these viruses has been proposed as a promising strategy. *Chiococca alba* (L.) Hitchc is a plant used by Yucatec Mayas traditional healers, mainly, as antipyretic and antirheumatic agents. To evaluate the potential of C. alba methanolic extracts against CHIKV and MAYV, preliminary analyses were carried out in vitro and in silico. The cytotoxicity profile of two C. alba roots methanolic extracts on Vero cells was performed by lysosomal viability analysis, using the neutral red assay, while the antiviral potential was determined by plaque assay. We further assessed, through in silico computational predictions using AutoDock Vina, possible interactions between the active site of the nsP2 proteases of these viruses with some secondary metabolites present in *C. alba* extracts, identified by High-Performance Liquid Chromatography (HPLC). Our partial phytochemical analyses revealed the presence of flavonoids and phenolic acids in the *C. alba* extracts. Our in vitro assays showed that both *C. alba* extracts inhibited more than 70% of CHIKV and MAYV activities at a concentration of 60 µg/mL. In silico analyses the flavonoids naringin and vitexin showed high ligand affinity to the protease enzyme nsP2 from CHIKV and MAYV, we, therefore, hypothesized that these bioligands may inhibit viral replication.

A4.R11 - Prospection of genes involved in biotic stress responses in the wild diploid species *Arachis stenosperma* and *Musa acuminata* Calcutta 4

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PTI and ETI plant defense mechanisms both activate metabolic pathways that culminate in responses that include secondary metabolism, oxidative burst, transcription factor activation and hormonal regulation. The characterization of defense genes and their expression during pathogen interaction is often complex. In addition, many genes involved in immune responses possess conserved domains, such that precise bioinformatic tools are required for accurate assembly of gene sequences and regulatory elements in these often-complex loci. The aim of this study is to validate candidate defense genes from sequences in libraries enriched for these in banana (*Musa* spp.) and peanut (*Arachis* spp.) wild resistant genotypes. Both cultures have high nutritional value, play a fundamental role in food security and, in commercial cultivars, are affected by a wide range of pathogens, including *Pseudocercospora musae* in banana and *Meloidogyne arenaria* in peanut. Candidate genes are being selected based on predicted function, based on differential RNAseq data for the host-pathogen interactions, as well as following RT-qPCR expression validation. Subsequently, genes are being tested for molecular and phenotypic effects in vivo

using an *Arabidopsis thaliana* plant model system. Candidate genes in resistant *M. acuminata* Calcutta 4 are being analyzed for complete gene sequences, to enable RT-qPCR expression validation during interaction with the pathogen *P. musae*, followed by cloning, transformation of *A. thaliana*, and validation in planta for function through over-expression and phenotype analysis during artificial pathogen infection. Candidate genes from resistant *A. stenosperma* are similarly being validated in *A. thaliana* challenged with the vascular wilt pathogen *Fusarium oxysporum* f. sp. conglutinans. Analysis of candidate genes will further our understanding of the adaptive response in *Musa* and *Arachis* and guide application in plant breeding for disease control.

A4.R12 - Identification and characterization of ligninolytic enzyme genes from a microbial unit enriched with lignin

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Lignin is nature's greatest source of phenolic compounds, unfortunately its recalcitrance to enzymatic conversion is a limiting step to increase its industrial value. An interesting factor in the use of this compound is that in the biorefinery models it is a renewable raw material that is not competitive with the food industry. Although bacteria are capable of degrading lignin in nature, most studies have focused on the degradation of lignin by fungi, so this work focused mainly on bacteria. A metagenomic library was assembled via natural selection over the time, obtaining microbial consortia enriched with lignin for a period of 12 weeks for microorganisms capable of using this compound as the only source of carbon. The metagenome was sequenced, and the best sample data were chosen for the work. The total of 232 genomes were recovered, their completeness and level of contamination were estimated and then refined. A total of 39 Metagenome-assembled genomes (MAGs) were selected for the analysis segments. Analyzes were performed via annotation of genes that encode proteins and phylogenetic relationships. 4 of these MAGs were chosen for analysis of metabolic fluxes according to their respective genomes. The choice of these was due to the high completeness and low contamination, the methodology used for the taxonomy was not able to go deeper, reaching only the phylum level.

A4.R13 - Heterologous expression of an antibody fragment in *Saccharomyces boulardii* for the treatment of colitis in mice

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Inflammatory bowel diseases (IBD) are chronic disorders such as Crohn's disease and Ulcerative Colitis characterized by the recurrent inflammation of the mucosa in defined areas or all throughout the colon. The incidence of such diseases are becoming more prevalent around the globe being associated with the process of industrialization, urbanization and increase in the consumption of ultra-processed foods. Treatments are generally carried out with the use of anti-inflammatory drugs, monoclonal anti-bodies (MAbs) and probiotics; *Saccharomyces boulardii* is a probiotic strain of yeast commercially used for treatment of diarrheas, with multiple studies indicating its capacity to modulate immune response locally and systemically. The advance of recombinant DNA technologies corroborate to the development of heterologous expression of MAbs by probiotic strains facilitating delivery of bioactive therapeutics to the site of inflammation while allowing anti-inflammatory modulation. In this research, genetically modified *Saccharomyces boulardii* expressing anti-CD3 monoclonal anti-bodies conjugated to the outer membrane was used for therapeutic treatment of mice challenged with Ulcerative Colitis induced by Sodium Dextran Sulfate.

A4.R14 - Development and application of biomembranes based on natural latex associated with phototherapy for the treatment of wounds in diabetic rats

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Introduction: Diabetic foot is a serious chronic complication of diabetes due to the complexity of pathophysiological mechanisms. Lower limb injuries can progress to amputation. Thus, the development of new therapies is needed. The objective was to develop and characterize natural latex biomembranes (BLN) with multilamellar liposomes (MLV) containing curcumin (CUR) and papain (PAP); and evaluate the effects in association with LED therapy on healing. Methodology: BLN were developed after drying a latex:liposome solution for 24h at 40 °C. The experiments were performed with 45 male Wistar rats with diabetes. The animals were randomly divided into experimental groups, control groups; untreated diabetic rats and groups that received treatment with isolated latex + LED; and the latex biomembrane group (PAP + LED (4) latex isolated + LED and (5) latex with CUR and PAP + LED. The treatment was based on LED therapy (Rapha® system) and application of BLN in the wound, the LED exposure was performed for 10 minutes, every 48 hours for 11 days. The progression of healing was calculated by comparing changes in wound diameters. Results: The MLVs had a varying size of 905.5 ± 122.3 nm, a polydispersion index of 0.764 and a surface charge of -40.7 ± 2.22 Mv. There was no change in shape and lamellarity when compared to the empty formulation. The BLN containing CUR and CUR and PAP, presented a transparent aspect, facilitating the irradiation. During treatment, faster healing was observed in the animals in the groups receiving CUR treatment with 99% wound contraction and 95% for the CUR / PAP group. Conclusions: The results have healing potential and are associated with the positive effects of phototherapy, latex and biocompounds, due to their different biological properties.

A4.R15 - Construction of infectious clone of cucurbit aphid-borne yellows virus brazilian melon isolate

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The Northeast region is the largest melon producer in Brazil, contributing about 90% of the national production. Brazil occupies the 11th place in the world ranking of melon production, being this fruit one of the most exported in recent years in the country. The main disease of melon crop in Brazil is caused by virus called “Amarelão do meloeiro”. This disease is associated with the carlavirus (Betaflexiviridae) Melon yellow-associated virus (MYaV) and the polerovirus (Luteoviridae) Cucurbit aphid-borne yellows virus (CABYV). CABYV was detected by the Next Generation Sequencing in Brazil in 2018. CABYV genomic RNA is about 5.7kb and encapsidated in icosahedral particle. The symptoms caused by solely CABYV in melon are not well-elucidated due to the mixed infection with MYaV. Therefore, the objective of this work was to construct an infectious CABYV clone. Total RNA was extracted from M3 isolate of CABYV using silica-based nucleic acid extraction protocol. The complete CABYV genome was amplified in two fragments by RT-PCR. For this, cDNA was synthesized with random or specific reverse primer using SuperScript IV reverse transcriptase. The 5' and 3' region fragments were amplified and, then, cloned and joined by Gibson Assembly using pJL89 in E. coli (DH10B). The construct was cloned into *Agrobacterium tumefaciens* (GV3101). The sequence of clones obtained from *E. coli* and *A. tumefaciens* was confirmed by Sanger sequencing. To evaluate the infectious clone, CABYV clones were agroinoculated in melon plants and the infection was evaluated by RT-PCR 10 days post-agroinoculation (dpa). Strong chlorosis symptoms were observed in all *N. benthamiana*

leaves. In melon plants, symptoms of internerval chlorosis were observed 10 dpa. CABYV was detected by RT-PCR with specific primers in all plants evaluated in the experiment. In conclusion, the complete CABYV-M3 isolate genome clone obtained from the Gibson Assembly methodology is infectious.

A4.R16 - Comparative analysis between extracellular components secreted by *Fonsecaea* sp isolates from different culture conditions in modulating the host's innate immune response

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Chromoblastomycosis (CBM) is a chronic cutaneous and subcutaneous mycosis, caused by black, dimorphic and filamentous fungi of the Dematiaceae family. *Fonsecaea pedrosoi* is considered one of the main species causing the disease in Brazil. Virulence factors produced by fungi, such as extracellular molecules present in the conditioned medium (CM) and extracellular vesicles (EVs) have been more studied in recent years, although there is a lack of information about the role of these molecules during CBM. This work aims to understand the in vitro and in vivo immunostimulatory patterns caused by molecules present in CMs obtained from *F. pedrosoi* and *F. erecta* conidia and hyphae cultivated in a rich medium (RM) and in a minimal medium (MM). The results showed that CMs isolated from MM are the most immunostimulatory, with increased secretion levels of TNF and IL-1β in vitro compared to CMs obtained from RM which have demonstrated to inhibit these cytokines production under certain conditions. The CMs obtained by *F. pedrosoi* and *F. erecta* were also used as an in vivo treatment and were shown to aggravate the progression of CBM in a murine model, increasing the diameter of the lesion area, the number of colony-forming units and the levels of pro-cytokines (TNF and IL-1β). It has already been observed that molecules present in the CM obtained by the capsular form of *C. neoformans* can inhibit the activation of the NLRP3 inflammasome and promote cell proliferation in vitro, indicating that the molecules present in the CMs of pathogenic fungi have the potential to immunomodulate the host's immune response. Thus, it is observed that CMs obtained by different species of the *Fonsecaea* genus have the ability to both stimulate and inhibit the secretion of important cytokines in the innate immune response depending on the nutritional conditions, demonstrating that they are molecules with the capacity for virulence and possibly to modulate the progression of CBM in humans.

A4.R17 - Horizontal gene transfer mediated by pCF10 plasmid in *Enterococcus faecalis*: Impact on the spread of antibiotic-resistant strains and evolution of virulence

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E. faecalis is a gram-positive, multidrug-resistant bacterium that has a vast capacity to transfer resistance genes, largely through the conjugation of plasmids responsive to pheromones, with pCF10 being one of the most studied so far. Objective: To analyze the initiation of conjugation mediated by the plasmid pCF10, as well as the plasmid regulation and phenotypic systems of gene transfer related to increased virulence and antibiotic resistance in *E. faecalis*. Conclusion: The pCF10 plasmid carry genes that encode antibiotics resistance and virulence factors inter and intra bacterial species, particularly, increasing the risk of *E. faecalis* infections, and the emergence of multi-resistant microorganisms.

A4.R18 - Transcriptional modulation of the GmEXPA gene via CRISPR/dCas9 technology aiming to increase soybean tolerance to phytonematodes

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Soy is one of the most important commodities, however, its cultivation has been impacted by the presence of phytonematodes that cause billions of dollars in damage. Nematotoids are the most abundant animals on Earth, they are morphologically simple and the phytonematodes belong to the genus Meloidogyne and are the ones that most impact both the quality and the yield of these cultivars. To combat these phytopathogens, producers usually use chemical nematicides, planting resistant cultivars and crop rotation, however, the use of nematicides causes impacts on the environment and cases of resistance to nematicides have already been reported. A class of proteins called expansins are involved in a range of developmental processes in land plants. Literature reports report that the overexpression of a class of these expansins (α-expansin) in model plants (*Arabidopsis thaliana* and *Nicotiana tabacum*) is associated with an increase in tolerance to *Meloidogyne incognita*. Thus, the aim of this project is to modulate the expression of the GmEXPA gene via the CRISPR-dCas9 system, aiming to increase the tolerance to the phytonematoid *M. incognita* in soybean plants.

A4.R19 - Detection of Bean-associated cytorhabdovirus (BaCV) Genes in Common Bean and *Bemisia tabaci* MEAM1

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Viruses are transmitted by whiteflies by different transmission modes. The carlavirus cowpea mild mottle virus is a nonpersistent virus. Criniviruses, torradoviruses, and ipomoviruses are semipersistent viruses and the transmission of poleroviruses by *Bemisia tabaci* is still unknown. Begomoviruses are persistent circulative viruses, but there is evidence that tomato yellow leaf curl virus replicates in the whitefly salivary glands. Plant rhabdoviruses are transmitted by their arthropod vectors in a persistent, circulative-propagative manner and they infect gut and muscle cells, nervous tissue, hemocytes, tracheae, and salivary glands. Bean-associated cytorhabdovirus RNAs corresponding to N, P, P3, P4, M, G genes were amplified from common beans and whiteflies collected in infected plants. Total RNA was extracted from 100 mg of leaves using the TRIzol Reagent. Total RNA was also extracted from a pool of 30 whiteflies using one-fourth of the volumes of the reagents in the standard protocol. RNAs were treated with TURBO DNase to eliminate any DNA trace. The cDNA was prepared with Anchored Oligo (dT) 20 primer and SuperScript III Reverse Transcriptase. The PCRs were performed with specific primers for all BaCV genes. The transcripts for Actin-11 (act11) and the small Rubisco subunit (RbcS) genes from common bean, and Ribosomal protein L9 (RpL9) and Vacuolar ATPase (v-ATPase) subunit A genes from whitefly, were used as internal reference controls and to identify possible transcripts ingested by the insects. BaCV ORFs were amplified, except for L probably because of its size (~6kb). Amplicons corresponding to act11 and RbcS were only amplified from the bean plant, and v- ATPase and RpL9 from whiteflies. It remains to be determined whether BaCV replicates in whiteflies, as observed for other rhabdoviruses and the assessment of dissected organs could facilitate its visualization by microscopy and/or by in situ hybridization.

A4.R20 - Characterization of the microbial consortium and identification of enzymes with lignolytic biotechnological potential

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Brazil is a major producer of plant biomass, as most of the national economy comes from agricultural products. Plant residue has components such as cellulose and hemicellulose of great interest and commercial value.

However, plant biomass still has a third component, lignin, which is the largest source of phenolic compounds in nature. However, due to its diverse molecular structure, it has fewer industrial applications. Therefore, lignin ends up having a less than noble destination. Using a biorefinery model, it is possible to add value to lignin by producing lignin-derived bioproducts. Microorganisms have digestive enzymes that are capable of degrading them and allowing the best use of lignin-derived bioproducts during the degradation process. This work aims to select a consortium specialized in lignin utilization, to identify specific microorganisms, as well as to explore their enzymatic potential for lignin degradation. The consortia were obtained from different soils, cultivated at 30 °C and 37 °C, enriched with different lignins (kraft or extracted by alkaline method) during six enrichment passes. The genomes were assembled from a total of 47,640,829 reads. From the soil samples, 232 genomes were recovered, and the Proteobacteria phylum is the most abundant of the three soils evaluated. Then the representation of the phyla: Actinobacteria, Firmicutes, Bacteroidetes, and Planctomycetes respectively. Of this total, only 29 were selected for functional analyzes as they showed completeness greater than 70% and contamination less than 10%. Alternatively, the clones from the metagenomic library are being functionally selected using guaiacol as a substrate. Twenty clones out of a total of 1000 from a first screening have already been pre-selected. These twenty will be re-evaluated to confirm the reaction to guaiacol. Later, these clones will be sequenced for annotation of ORFs and it is possible to infer which ORF is responsible for the phenotype.

A4.R21 - Bioprospection and genetic engineering of fungi for synthesis of high value-added products

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The use of microorganisms for the production of biomolecules, whether naturally or by means of genetic engineering, is of great, and increasing, commercial interest. Lovastatin is a molecule produced naturally by some fungi, including *Aspergillus terreus*. It acts as a competitive inhibitor of a key enzyme in the cholesterol synthesis pathway, 3-hydroxy-3-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase, and thus is used as a drug against hypercholesterolemia. Given the great biodiversity of Brazil, aspergilli isolated in this country were evaluated for their genomic diversity, phylogenetic relationship and lovastatin production. We thus identified a promising strain, URM 5961 that produces 0.6 g/L of lovastatin and has a high degree of genomic identity with the reference strain, ATCC 20542, which produces about 1.0 g/L. The hyaluronic acid (HA), a biopolymer produced naturally by animals and some pathogenic microorganisms. Due to its rheological characteristics such as high viscosity and biocompatibility, HA is widely used in medical and ophthalmic treatment, facial filling procedures and in the formulation of cosmetics. In light of that, we also investigated the potential of the yeast *Ogataea polymorpha* as a

host for the production of HA. To this end, three strains were created, EMB 102, EMB 103 and EMB 104, which differ in terms of the promoters used to confirm the ability of O. polymorpha to produce HA. The EMB 103 strain achieved an HA titer of 0.8 mg/ml. The 2'-fucosylactose (2'-FL) corresponds to about 80% of the oligosaccharides present in human maternal milk. It stands out for its positive immunomodulatory activity in newborns, and is considered a potential additive in infant formulas. Thus, in the yeast *Kluyveromyces lactis* was by introduction of the genes encoding the enzymes mannose 4,6-dehydratase (gmd), GDP-L-fucose-synthase (fcl) and a putative fusosyltransferase (wcfB), generating the K2W strain, which is in the validation phase of 2'-FL production.

A4.R22 - Prospecting of ruminal bacteria and evaluating their role in lignin deconstruction

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Lignin is an aromatic macromolecule composed mainly of monomers: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol. It is part of the plant cell wall corresponding to 15-30% of its constitution. This biomass fraction is one of the main contributors to the recalcitrance of plant biomass, and in many industrial processes, its removal is necessary for the use of cellulose and other fractions to obtain bioproducts. However, lignin represents a renewable source of aromatic compounds that can be used to produce products of industrial interest, such as vanillin, guaiacol, and phenol. A central step for the valorization of lignin is its deconstruction, and the use of bacteria has attracted increasing attention due to its ability to adapt to different environments and biochemical versatility. The bovine rumen is a microaerobic environment that hosts a diverse microbiota, and bacteria are highly specialized in the degradation of lignocellulose. In this sense, samples from the bovine rumen were inoculated in culture media containing kraft lignin with or without yeast extract as a carbon source at 37 °C for four days, under anaerobic conditions. The communities were able to decolorize and degrade the lignin present in the culture media, where the maximum discoloration for the culture grown on kraft lignin and yeast extract as a carbon source was 39%, and 35% for the culture obtained using kraft lignin as a carbon source. Lignin degradation reached values of 34% and 30% for cultures containing kraft lignin/yeast extract and kraft lignin as a carbon source The taxonomic analysis of these communities (based on 16S rRNA) indicated the enrichment of members of the Enterococcaceae family, belonging to the Proteobacteria phylum. Furthermore, the GC-MS analysis of the culture supernatants detected the presence of several compounds of biotechnological interest, such as phenylacetic, 3-phenylatic acids. Thus, indicating the potential of these communities to degrade lignin.

A4.R23 - Plume layer impacts on the Amazon reef sponge microbiome: primary producers

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Symbiont relationships between corals and photosynthetic microorganisms sustain coral reef existence. However, the Amazon Reef stays under a plume layer that attenuates the entry of light and instead of corals sponges are the major reef metazoan. We used Metagenome-Assembled genomes to investigate how the sponge microbiome supports its host and overcomes the reduced light availability. METABOLIC software profiled metabolic and biogeochemical functional traits of the microbiome. We recovered 248 bins from Agelas and Geodia sponges with completeness > 70% and contamination < 10%. Genome coverage revealed differences between the microbiome composition of Agelas and Geodia; Chloroflexota taxa were the most abundant in Agelas and Thermoproteota taxa

in Geodia. Gene coverage and metabolic profiles revealed photosynthetic apparatus absence in the microbial community. Carbon fixation pathways: 3-Hydroxypropionate/4-Hydroxybutyrate Cycle, 3-Hydroxypropionate Bicycle, Reductive Tricarboxylic Acid Cycle, and Calvin-Benson-Bassham Cycle were present with low gene coverage. Amino acid utilization and fermentation were the most abundant microbiome functions. The metabolic profile shows that the Amazon reef sponges host microorganisms involved in the nitrogen cycle, nitric oxide reduction (norBC) were the most abundant; other nitrogen functions detected were: ammonia oxidation, nitrate reduction (napAB, narGH), nitrite ammonification (nirBD, nrfADH), nitrite oxidation, and nitrite reduction (nirKS), but in lower abundance. Metabolic profiling and gene coverage showed enrichment of sulfate reduction and sulfite oxidation functions. The presence of the Amazon plume limits photosynthetic microorganism metabolism. Chemoautotrophs are present but seem to play an auxiliary role rather than the principal source of organic matter. Possibly, nutrients from the Amazon River support sponges because they can filter through the water and are less dependent on carbon-fixing microorganisms.

A4.R24 - Dynamic interactome of *Trypanosoma cruzi* RBPs and RNAs during infection

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Chagas disease is a potentially fatal, chronic, and systemic pathology caused by the protozoan *Trypanosoma cruzi*. This illness is endemic in Latin America, but intense population migratory movements have contributed to its distribution to non-endemic countries. World Health Organization estimates that 6 to 7 million people are infected worldwide. The drug available to treat it, benznidazole, has low efficacy in the chronic phase, in addition to strong side effects, requiring the development of new medicines. Challenges faced for this development are the high complexity of biological and interaction processes of the parasite with the host cell. The various phenotypic changes and adaptations observed throughout its cycle and during the infection process depend on extensive modulations of gene expression. In this context, RNA-binding proteins (RBPs) stand out, which play a crucial role in regulating several cell processes, such as mRNA stabilization, mRNA degradation, and the protection of transcripts by recruitment into granules. Therefore, the present project aims to study the interactions between RBP-RNAs in the initial moments of infection in a host cell to elucidate and assess the importance of these interactions and their constituents in this crucial disease establishment process and maintenance. For this purpose, the strategy is to perform in vitro infection assays in human cells with the OOPS methodology (Orthogonal Organic Phase Separation), recently published, for stabilization and purification of RBP-RNA complexes, and the separation of its components for proteomic analysis by mass spectrometry and transcriptomic by RNA sequencing. For the differentiation of hosts and trypanosomatids proteins, the Stable Isotope Labeling by Amino Acids in Culture method (SILAC) will be used in mammals cells cultivation. The expectation is to elucidate possible interactions between RBP-RNAs, and it's importance to the infection process.

A4.R25 - In silico structural analysis and biochemical characterization of thimet oligopeptidase from *Trypanosoma cruzi*

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Chagas is a neglected disease affecting approximately 7 million people worldwide. Little or no progress has been made towards the development of safe and effective treatments for the severe cases of the disease. Parasite proteases are promising drug target alternatives, given their central roles in essential biological processes. Thimet Oligopeptidase (TOP) is a 77,5kDa metallopeptidase belonging to the M3A family. Little is known about TOP from tripanosomatids, although it has been identified in the subproteome of *Trypanosoma cruzi.* Here, we performed in silico analysis of TOPTc and in vitro enzyme assays in order to characterize the main biological properties of the protease. Also, Anti-TOPTc polyclonal antibodies produced in mouse were used for cytolocalization using fluorescence microscopy. Structural predictions showed that TOPTc has a zync catalytic domain in positions 474 and 478 and the active site is located on the position 476. The protein sequence is not predicted to possess signal peptide nor transmembrane domains. Although no tertiary structure has been resolved for TOPTc, molecular model from AlphaFold yielded good parameters scores, indicating it could be used for further analysis. Recombinant TOPTc was expressed in E.coli and purified by affinity-chromatography. Maximum activity of the enzyme was obtained with phosphate buffer pH 7, 5µM ZnCl2. Km for the specific substrate Mca-Pro-Leu-Gly-Pro-D-Lys is 24,82mM. The enzyme is still active in high temperature (60°C – 80°C), which could be explained by its 30% sequence identity with archaea metalopeptidases. 1 – 10mM EDTA increases TOPTc activity while phenanthroline (IC50 0,372mM) completely inhibits enzyme activity. Specific inhibitor Cpp-Ala-Ala-Phe has an Ic50 of 54,29nM. Immunocytolocalization was performed with polyclonal antibodies produced in mouse and resulted in identification of the protein spread throughout the parasite.

A4.R26 - Epidemic or Pandemic by SARS-CoV-2 and the future of humanity

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Life after the SarS-2 pandemic, what to expect for the future of humanity? During the course of mankind's development, the global population has experienced various health crises similar to that of COVID-19. At certain times, different crises have occurred, consequently caused by different infectious agents, such as the Black Death in the 14th Century, which caused about 200 million deaths, and the Spanish Flu in the 20th Century, which caused about 100 million deaths. For Yuval Noah Harari, the pandemic that started in 2020 is nothing compared to those others. "In the war between humans and pathogens, humans have never been stronger." The present study aimed to review the literature regarding the SARS-CoV-2 epidemic or pandemic and the future of humanity. The emergence and reemergence of viral pathogens increases with the intense geodynamic activity of the planet. The predictable and current Severe Acute Respiratory Syndrome- associated Coronavirus type 2 (SARS-CoV-2) pandemic, given the evolution of low pathogenicity coronaviruses to more aggressive forms, such as Severe Acute Respiratory Syndrome-associated Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS), is worrisome, given the high incidence of deaths among the elderly and individuals of any age with comorbidities, debilitated, triggering government measures that are leading to worldwide social and economic collapse. Statistically treated data has shown that the death rate from SARS-CoV-2 infection in patients who developed COVID-19 is variable from region to region, taking into account climate, ethnic composition, economic development, population density, and more. The data from the brief survey show that there is still much more to know about COVID-19. However, all the lessons we have learned in the past from the SARS and MERS epidemics are the best cultural weapons to face this new global threat.

A4.R27 - Metabolic engineering of *K. phaffii* for L-lactate production in aerobiosis from crude glycerol

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L-lactic acid is a platform chemical that can be polymerized into poly-lactic acid (PLA). It is a product of growing industrial interest due to its diverse applications, with emphasis on its application for the production of biodegradable bioplastics. Its production over the years comes from the fermentation by lactic acid bacteria or by chemmical synthesis. However, both routes have the disadvantage of producing a racemic mixture of the two optic isomers. For this reason, heterologous production in yeasts can be an alternative because it presents the possibility of expressing the gene of a lactate dehydrogenase enzyme that results in only one optic isomer, thus reducing purification costs. For this reason, the yeasts *Komagataella phaffii* was chosen for the L-lactate production due its ability to consume raw glycerol, an important residue of the biodiesel industry. Furthermore, crude glycerol may contain traces of methanol, which can be toxic for some yeats. However, in response to its methylotrophic ability, the presence of methanol is not harmfull for *K. phaffii.* Indeed, it can be considered another advantage of this yeast. Throughtout the years, some strains of *K. phaffii* have been engineered to produce L-lactate with a yield of up to 67% in the conversion of glycerol into L-lactate. Therefore, the aim of this work is to improve the already constructed strains of *K. phaffii* by the disruption of the gene responsible for the mitochondrial pyruvate transporter, mpc1, in the GLp strain, in order to generate the GLpm strain. The integration of the cassette is expected to disrupt the mpc1 gene and hygromycin will be used as selection marker. Thus, we expect to be able to contribute to the analysis of the synergistic effect of the pdc/mpc double deletion to increase the pool of pyruvate in the cytosol and whether this factor is sufficient to increase the yield of L-lactate production.

A4.R28 - Description of the nutritional profile of microbiomes associated with marine sponges from Antarctica and Chilean Patagonia

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Sponges appeared 600 million years ago. At this time, bacteria were established in the oceans which means that sponges evolved in a sea of potential symbionts. The three domains of life reside in sponges and the symbiont community represents 40% of its biomass. In Antarctica, a geographically isolated ecosystem, sponges are a attractive model for studying symbiosis. This work aimed to characterize the nutritional profile of sponge microbiomes. For this, 59 individuals in Antarctic and 12 in Patagonia were collected. DNA sequencing was performed using the V4 region of the 16S rRNA gene and sequenced on the Illumina Miseq platform. The taxonomic classification of microorganisms was attributed to ASVs obtained from bioinformatics analysis. The 30 most abundant ASVs of each individual were classified as guild. Altogether 18 sponge species were analyzed: *T.enuicapitata, C. retamalesi, D. antarctica, Haliclona (Rhizoniera)* sp, *Haliclona (Soestella)* sp, *H.torquata, I. radiatus, I. unicorne, I. kerguelenensi, I. toxophila, M. acerata e M. lissostyla, from Antarctica, and H. attenuata, Iophon* sp, *M. magellanica, Clathria (Axosuberites)* sp, *cf. Topsentia sp e Petrosia (Petrosia)* sp, from Patagonia. The number of sequences found was of 2.221.250 and the total of ASVs found was of 6.195. The microbiomes were dominated by heterotrophics, mainly Proteobacteria. All species from Antarctica, except *C. retamalesi*, presented chemoautotrophic, as well as *Iophon* sp and *M. magellanica* from Patagonia. Chemoautotrophics were mainly

represented by bacteria of SUP05 clade. Phototrophics were found in all species of Patagonia, except *Clathria (Axosuberites)* sp and *Petrosia (Petrosia)* sp. And only *Haliclona (Soestella)* sp, in antarctica. Most phototrophics were represented by cyanobacteria. Electroautotrophics represented by the genus *Candidatus Tenderia* sp were found in Antarctic species. Comparing genus of porifers present in both places, we found that it is possible to use the presence of phototrophs and archae to distinguish the porifers species between them.

A4.R29 - Multi-omic analysis of *Corynebacterium glutamicum* during glutamic acid overproduction stimulus

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Corynebacterium glutamicum is a bacterium capable to produce a broad variety of industrial relevant biomolecules, especially amino acids. Industrial amino acid production is a growing market with annual production of approximately 2.5 million tons. Previous reports have evidenced metabolic regulation related to glutamic acid overproduction in *C. glutamicum* to proteins post-translational modifications and transcript regulation. Multi-omics approaches are rising in the discovery of new mechanisms and pattern recognition of microbial organisms of industrial interest. This analysis has capacity to identify metabolic regulation in different molecular levels. Despite the economic and biotechnological relevance of *C. glutamicum* to the production of amino acids, the process of glutamic acid production remains elusive. This project uses the innovative approach of multi-omics analysis in order to clarify new mechanisms and comprehend the glutamic acid production of *C. glutamicum*. In a pilot study, *C. glutamicum* was grown in CGXII medium, and glutamic acid production was stimulated by addition of tween 40 (4mg/mL) after 6 h. Then, 30 hours later, the extracellular was submitted to amino acid analysis, meanwhile the intracellular fraction was analyzed by bottom-up and top-down proteomics. This preliminary study demonstrated the glutamic acid production through tween-40 stimulus. However only 0.16 g/L of glutamic acid were produced after 36 h. Nevertheless, proteomic analysis revealed several regulated proteins and enriched pathways, such as biosynthesis of amino acids, carbon metabolism and biosynthesis of secondary metabolites. Furthermore, proteins related to membrane and cell wall metabolism presented fold change > 5, suggesting relevance of this mechanism in the production of glutamic acid. Qualitative top-down proteomics identified some of regulated proteins related to cell wall metabolism and amino acid biosynthesis with undescribed proteoforms.

A4.R30 - Prospection of Enzymes from Enriched Ruminal Microbiome for Lignin Depolimerization

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Sustainable energy based on renewable resources are required to meet the world's future energy needs. That is why lignocellulosic biomass is attracting the attention. Lignocellulose is mainly formed by sugars such as cellulose and hemicellulose, and lignin which is a matrix rich in phenolic compounds. The lignocellulosic biomass is a source of a variety of molecules. Lignin is a abundant residue from agricultural and industrial activities. Lignin´s molecules reutilization is tough due to its recalcitrance. Bacteria can secrete ligninolytic enzymes. Among the main enzymes there are Laccases, Lignin Peroxidases, Manganese peroxidase and Versatile Peroxidases. Another report showed that some strains can utilize lignin derivates and convert them into smaller phenolic compounds. Recently lignin has been used to generate energy through combustion, but its conversion into high-value chemicals is more interesting. Omics approaches contributed to identify enzymes, pathways, and products from lignin depolymerization. This work aims to discover novel bacteria, enzymes and pathways in which lignin is processed.

Anaerobic bacteria have shown to produce lignin-degrading enzymes, and there is few information about anaerobic lignin breakdown in the literature. After obtaining a consortium capable of growing on cellulose from bovine rumen, we isolated bacteria capable of growing on mineral media containing kraft-lignin as the only carbon source. These bacteria are believed to retain in their genome the sequences for lignin-active proteins. We performed the growth of these bacteria in kraft-lignin media for DNA extraction and sequencing, to identify taxonomic affiliation and genes involved in the metabolization of lignin. Some isolates grew on Kraft Lignin, which indicates that they are capable of lignin breakdown. Three isolates decolorized the lignin media and were selected for sequencing.


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