2nd INTERNATIONAL WORKSHOP

On Applied Cellular and Molecular Biology

Abstract's book

UNIVERSIDAD DE LA FRONTERA

DOCTORADO BIOMOL

II International Workshop on Applied Cellular and Molecular Biology

January 24 - 25, 2023

UNIVERSIDAD DE LA FRONTERA

DOCTORADO BIOMOL

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2nd INTERNATIONAL WORKSHOP

On Applied Cellular and Molecular Biology

Programme

Tuesday January 24

Main Room	**Opening Ceremony**
08:30 - 08:45	**Welcoming**
08:45 - 09:15	**Opening Conference:** **Dr. Alejandra Chaparro Padilla - Universidad de Los Andes, Chile.** "Oral Precision Medicine. New Perspectives"
Main Room	**Symposium 1: Plant Physiological and Molecular Responses to Environmental Stress** Co-Chairs: Marjorie Reyes Díaz - León Bravo Ramírez
09:20 - 09:50	**Dr. Paulo Ribeiro Marchiori, Universidade Federal de Lavras, Brazil.** "Comments on physiology of sugarcane ripening: carbon partition"
09:55 - 10:25	**Dr. Adriano Nunes-Nesi, Universidade Federal de Viçosa, Brazil.** "Mitochondrial metabolism and plant stress responses"
10:30 - 10:40	**Break**
10:40 - 11:10	**Dr. Helaine Carrer, Universidade de Sao Paulo, Brazil.** "Molecular and biotechnology approaches to improve water deficit tolerance in teak trees"
11:15 - 11:45	**Dr. Arnould Savouré, Sorbonne Université, France.** "Insights into the roles of proline in plants"
11:50 - 12:05	**Madeleyne Parra-Fuentes, Corporación Colombiana de Investigación Agropecuaria, Colombia.** “Diagnosis and characterization of symptoms associated with citrus Huanglongbing disease in the Colombian Caribbean”
12:10 - 12:25	**Kaík Faria de Sousa, Universidad Federal de Viçosa, Brazil.** “Relationship between the high photosynthetic efficiency and leaf anatomic traits in *Solanum pennellii* introgression sublines”
12:30 - 12:45	**Dariel López Hernández, Universidad de La Frontera, Chile.** “How nocturnal warming could lead to *Deschampsia antarctica (Poaceae)* cold deacclimation?”
Main Room	**Symposium 2: Biotechnology Applied to Health** Co-Chairs: Pamela Leal Rojas - Luis Salazar Navarrete
14:30 - 15:00	**Dr. Katlin Brauer Massirer, Universidade Estadual de Campinas, Brazil.** “Development of enzymes for viral detection by RT-LAMP”
15:05 - 15:35	**Dr. Leticia Barrientos Díaz, Universidad de La Frontera, Chile.** “An innovative multi-omics approach for bioactive metabolites discovery in actinobacteria and cyanobacteria from extreme environments”

15:40 - 15:50	**Break**
15:50 - 16:05	**Nivaldo Gómez Hernández, Center for Genetic Engineering and Biotechnology, Cuba.** “New evidence of the interaction between the COMMD1 protein and the antitumor peptide CIGB-552”
16:10 - 16:25	**Álvaro Gutiérrez Colima, Universidad de La Frontera, Chile.** “Bacteriocins and its potential effect as cell death mediator in colon cancer cells lipid membrane: An *in-silico* analysis”
16:30 - 16:45	**Felipe Maurelia Gaete, Universidad de Concepción, Chile.** “Identification and characterization of proteic interactors for the C-terminal region of SCO-spondin during embryo development”
16:50 - 17:05	**Ivonne Romero Aguilera, Centro de Protección e Higiene de las Radiaciones, Cuba.** “Chromosomal radiosensitivity in lymphocytes of cancer patient measured after G0 *in vitro* irradiation and G2-checkpoint abrogation by caffeine”
17:10 - 17:25	**Nicole Cortez Salvo, Universidad de La Frontera, Chile.** “Biotransformation products of drimane sesquiterpenoids with activity against pathogenic yeast”
17:30 - 17:45	**Benjamín Leyton-Carcaman, Universidad de La Frontera, Chile.** “The clinically relevant *Corynebacterium* spp insertion sequences determine their role in resistance and virulence”
17:50 - 18:05	**Johan Morales-Sánchez, Instituto Tecnológico de Costa Rica, Costa Rica.** "In-vitro anticancerogenic activity preliminary tests for a Costa Rican species of Ganoderma"
18:10 - 18:25	**Paula Marroquín Morales - Daniela Suárez Bernal, Instituto Tecnológico y de Estudios Superiores de Monterrey, Mexico.** "Optomotor response (OMR) more than a visual test. Modeling diseases of national interest"

Programme

Wednesday January 25

Main Room	**Symposium 3: Cellular and Molecular Biology of Reproduction** Co-Chairs: Pamela Uribe Catalán - Ricardo Felmer Dorner
09:00 - 09:30	**Dr. Ricardo Perecin Nociti, Universidade de Sao Paulo, Brazil / Université de Montréal, Canada.** "LncRNAs in the embryo and developmental biology"
09:35 - 10:05	**Dr. María Elena Arias Cea, Universidad de La Frontera, Chile.** "Activation of bovine oocytes by protein synthesis inhibitors: New findings on the role of MPF/MAPKs"
10:10 - 10:20	**Break**
10:20 - 10:50	**Dr. Rodrigo Boguen Ojeda, Universidad Católica de Temuco, Chile.** "Effect of pathological Rho GTPase modulation on function and structure of human spermatozoa"
10:55 - 11:10	**Dr. (c) Fernanda Fuentes Zapata, Universidad de La Frontera, Chile.** "Mammalian gametogenesis and fertilisation: Main molecular events in germ cells"
11:15 - 11:30	**Dr. Osvaldo Merino Painen, Universidad de La Frontera, Chile.** "Effects of climate change on sperm function and motility kinematic parameters in sperm from fish"

11:35 - 11:50	**Dr. Fabiola Zambrano Quezada, Universidad de La Frontera, Chile.** "Neutrophil extracellular traps and their impact on sperm function"
11:55 - 12:10	**Luis Águila Paredes, Universidad de La Frontera, Chile.** "Effect of sperm treatment with lysolecithin on in vitro outcomes of equine ICSI"
12:15 - 12:30	**Paulina Cabrera Herreros, Universidad de La Frontera, Chile.** “Effect of incubation of thawed equine spermatozoa under different capacitating conditions on the sperm viability and membrane fluidity”
12:35 - 12:50	**Felipe Pérez García, Universidad de La Frontera, Chile.** "Isolation of small extracelular vesicles (<200 nm) from bovine follicular fluid by ultracentrifugation"
Main Room	**Symposium 4: Opportunities in Research and Applied Science** Co-Chairs: Priscilla Brebi Mieville - Jorge Farías Avendaño
14:30 - 15:00	**Mg. Franklin Valdebenito Godoy, Universidad de La Frontera, Chile.** "Innovation and entrepreneurship from the university"
15:05 - 15:20	**Dr. Bárbara Mora Lagos, Universidad Autónoma de Chile, Chile.** "New pharmacological formulation for the treatment of gastric cancer: a potential alternative in chemoresistance”
15:25 - 15:40	**MSc. Rodrigo Salazar Celedón, Universidad de La Frontera, Chile.** "Bactomelanin®: Exploiting the potential of bacterial pigments of Antarctic origin as photoprotectors against ultraviolet radiation"
Poster Room	**Poster Session**
15:55 - 17:00	**Poster Presentation**
Main Room	**Closing Conference**
17:10 - 17:40	**Mg. Carlos Isaacs Bornand, Universidad de La Frontera, Chile.** "Managing emerging technologies: New tools to move from lab to market"
17:45 - 18:15	**Awards and Closing Ceremony**

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PREFACE

This Book of Abstracts contains the formal publications of the **II International Workshop on Applied Cellular and Molecular Biology** organized by the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology of the Universidad de La Frontera, held on the 24th and 25th January 2023 (Temuco, Chile).

The **II International Workshop on Applied Cellular and Molecular Biology** will cover the following topics: 1) Plant Physiological and Molecular Responses to Environmental Stress, 2) Biotechnology Applied to Health, 3) Cellular and Molecular Biology of Priority Diseases, and 4) Opportunities in Research and Applied Science. The first three scientific topics together with the knowledge development translated into industrial innovative products and services are important cornerstones for the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology.

Our Doctoral Program was established in 2005 by the Universidad de La Frontera (Res. Ex. 0310 27/01/2005) because of the evolution and disciplinary experience generated in the University for over a decade at that time. Nowadays, the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology is one of the most recognized Doctoral Program in the field of Cellular and Molecular Biology in Chile. Its 116 alumni are currently contributing to the development of the country with basic and applied knowledge, in public and private organizations.

At a global level, cooperation involving students and academic staff have been developed over the last years, which has led to an increase in the number and quality of doctoral theses developed under both internship and double doctoral degree agreements with high quality institutions around the world. Also, foreign students from abroad have chosen Chile to develop their doctoral studies. In this context, this Doctoral Program has been selected by high quality foreign students to develop their doctoral theses, to develop an international doctoral internship or to develop their theses under a double doctoral degree. All these actions have had a positive influence on the increase in scientific productivity and, consequently, on the quality impact of generated publications and knowledge.

The II International Workshop on Applied Cellular and Molecular Biology is indeed a good opportunity to congregate altogether our students based in Chile, our students from abroad that have already developed or will develop their internship in our Doctoral Program, our academic staff, and our academic colleagues from national and foreign institutions. We are delighted at the participation of students, researchers, and professors from 11 countries (Argentina, Brazil, Canada, Colombia, Costa Rica, Cuba, France, Panama, Mexico, Spain, and Chile) and the more than two hundred attendants resulting in excellent seventeen keynotes, fourteen oral communications and twenty-eight posters. These numbers confirm the II International Workshop on Applied Cellular and Molecular Biology will be a significant international conference.

Thanks are due to the many individuals who have been involved at all levels of the planning, coordinating, reviewing, organizing, and convening of the symposia. The virtual meeting facilities and, last but not the least, the Agencia de Comunicaciones Xplain (Chile) are also acknowledged. Finally, we wish you a very productive meeting and hope to see you personally very soon in Temuco.

Organizing Committee

Opening conference

Oral precision medicine: New perspectives.

Chaparro, Alejandra.

Facultad de Odontología, Universidad de Los Andes, Santiago - Chile.

Several studies have proposed an association between periodontitis and chronic non-communicable diseases (NCDs), suggesting that periodontitis is a risk condition for those diseases, which could increase systemic inflammatory biomarkers and low-grade systemic inflammation, which are the suggested mechanisms involved in this association. Periodontitis is an immune chronic inflammatory disease in the periodontal tissues, with alveolar bone destruction, loss of periodontal attachment, and eventually leading to tooth loss. Besides, periodontitis is also related to a cardiovascular disease, poor glycemic control in diabetes, cognitive decline, and adverse pregnancy outcomes. The personalized precision approach, based on identifying biomarkers such as proteins, extracellular vesicles, genes, and non-coding RNAs, among others, is now an up-to-date research topic due to their diagnostic utility and the subsequent risk stratification of the population. A biomarker is a characteristic that is measured as an indicator of normal or pathological biological processes or responses to an exposure or intervention. There are several categories for biomarkers, including susceptibility risk, diagnostic, prognostic, pharmacodynamic/response, predictive, monitoring, safety, and surrogate endpoints. Susceptibility biomarkers identify risk factors, and prognostic biomarkers can predict disease trajectory, guiding prevention and stratifying patients, redefining disease categories to align with pathophysiology. Both, the early diagnosis in non-symptomatic stages, as well as the prediction of NCDs using oral biomarkers, combined with clinical variables of the subjects, represent a promising area of development, which will allow improving the characterization of periodontitis and their link with NCDs, advancing the diagnosis, preventing pathologies, and increase clarity concerning prognosis and monitoring of them. This initiative includes the study of biomarkers in oral fluids that allows the development of early diagnostic technologies based on the detection of sensitive biomarkers in liquid biopsies in a minimally invasive approach for prediction, early diagnosis, and monitoring of different systemic NCDs diseases related to periodontitis.

Symposium 1: Plant Physiological and Molecular Responses to Environmental Stress

Co-chairs: Dr. Marjorie Reyes Díaz and Dr. León Bravo Ramírez

Comments on physiology of sugarcane ripening: carbon partition.

Ribeiro-Marchiori, Paulo E.

Sector of Plant Physiology, Biology Department, Institute of Natural Sciences, Federal University of Lavras, Brazil.

The biomass accumulation in plants is result of several integrated biological processes along the plant lifespan, associated to positive carbon (C) balance. The C-balance is defined as the difference among the C input (mainly photosynthesis) and output (mainly cellular respiration). Considering crops, the harvest index (HI) is also an important trait for economic viability of the crop, which is determined by the C partitioning. Sugarcane (*Sacharum spp.*) is one of the main energetic crops, cultivated to produce sucrose, ethanol, and electric energy. However, between interception of light energy by the sugarcane leaves for photosynthesis and the final storage of sucrose in stalks before harvest, a very intricate series of molecular, biochemical, and physiological events occur, always dependent on the environmental conditions. During the 12 to 18 months the sugarcane crop takes to be suitable for harvest, the photosynthesis/respiration process and carbon partitioning events are modulated by the environment. Here we will discuss the variation of photosynthesis in canopy (main source) and the main destination of the assimilated carbon (sinks) along the growth season and how the environment induces changes in carbon partitioning in these plants. Therefore, it's important to understand how environmental conditions affect the C-balance in sugarcane plants to define strategies to mitigate the damages in sucrose yield due to stressful situations.

Mitochondrial Metabolism and Plant Stress Responses.

Nunes-Nesi, Adriano; Monteiro-Batista, Rita de Cássia; Fonseca-Pereira, da Paula; Araújo, Wagner L.

National Institute of Science and Technology on Plant Physiology under Stress Conditions, Departamento de Biologia Vegetal, Universidade Federal de Viçosa 36570-900 Viçosa, MG, Brazil.

In the future, CO2 concentrations, together with the temperature, are expected to be considerably higher than today. Drought periods are likely to be more frequent and severe, while flooding also increases, leading to soil degradation and increased incidence of climatic extreme events. Thus, understanding the impact of ongoing climate change on crop physiology and performance is essential for predicting future yields and ensuring food security. It has been demonstrated in plants that mitochondrial metabolism is integrated with other essential processes such as photosynthesis and photorespiration and is associated with plant responses to stress conditions. Some of these studies suggest that mitochondrial proteins participate in the regulation of stomatal opening and development, nitrogen metabolism, as well as changes in leaf redox potential. During my presentation, I will discuss the mechanisms by which mitochondria modulate growth and photosynthesis in plants under stress conditions.

Acknowledgments: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Molecular and biotechnology approaches to improve water deficit tolerance in teak trees.

Oliveira, P[1]; Hurtado, F[1]; Galeano, E.[2]; **Carrer, H[1].**

[1]College of Agriculture "Luiz de Queiroz", University of São Paulo, Brazil.
[2]Department of Forestry, Mississippi State University, USA.

Teak (*Tectona grandis L.*), tree native to Southeast Asia, is currently the preferred choice in the timber trade and agro-forestry in Latin America due to the wood high quality and properties. However, a robust understanding about genes related to drought stress in this species are not well known and are essential to elucidate the molecular biology and provide information for practical purposes. Thus, the objectives of this work were to determine new procedures for genetic engineering in teak including an efficient plant regeneration system and to explore the transcriptomic data of more than 400,000 clusters to identify genes related to water stress. Plant tissues such as foliar and internode were cultivated with different hormonal dosages in vitro to obtain better protocol for plant regeneration. In addition, the transcriptomic analysis returned 1,145 sequences, and from these TgTPS1, TgDREB1, TgAREB1, TgPIP1 TgHSP1, TgHSP2, TgHSP3 and TgBI (related to osmoprotection) were selected. RT-PCR expression analysis was performed in plants subjected to moderate and severe stress. Expression analysis of those genes showed that, in severe treatment only TgTPS1 and TgDREB1 presented higher relative expression than the control. For all genes studied, the relative expression in the control treatment was lower.

Acknowledgments: To FAPESP for research funding, Proteca Biotecnologia Florestal LTDA for the Partnership in the Project PITE/FAPESP 2015/50634-1 and CAPES for the scholarship to Perla Novais de Oliveira.

Insights into the roles of proline in plants.

Savouré, Arnould.

Institute of Ecology and Environmental Sciences of Paris (iEES), UPEC, CNRS, IRD, INRAE, Sorbonne Université, F-75005 Paris, France.

The amino acid proline has been known for many years to be a component of proteins as well as to play a role as an osmolyte in plants under various stress conditions. Many recent studies have demonstrated that proline has other roles. Proline accumulation is intimately connected to many cellular processes, such as osmotic pressure, energy status, nutrient availability, and changes in redox balance. Proline biosynthesis and catabolism is linked to photosynthesis and mitochondrial respiration, respectively. Proline can function as a signal, modulating gene expression and certain metabolic processes. In this talk, I will review important findings on proline metabolism and function of the last decade, giving a more informative picture about the regulation of the proline metabolism and the function of this unusual amino acid in maintaining cellular homeostasis, modulating plant development, and promoting stress acclimation

Diagnóstico y caracterización de síntomas asociados a la enfermedad Huanglongbing de los cítricos en el Caribe colombiano.

Parra-Fuentes, Madeleyne; Soto-Suárez, Mauricio; Lovera, Andrea; Gómez-Correa, Juan; Guzmán-Sánchez, Luisa; Pérez-Artiles, Lumey.

Corporación Colombiana de Investigación Agropecuaria, Agrosavia, Centro de Investigación Caribia.

Huanglongbing (HLB), enfermedad asociada a Candidatus Liberibacter asiaticus (CLas), se caracteriza por disminuir la productividad y causar la muerte progresiva de las plantas de cítricos. Con el propósito de disponer de métodos de diagnóstico rápidos a CLas como estrategia de monitoreo en campo de la enfermedad HLB, se evaluaron metodologías presuntivas como: inspección visual de síntomas y prueba de yodo (raspado y macerado), frente a la técnica molecular de amplificación isotérmica mediada por bucle o LAMP (AmplifyRP® Acceler8® for Las (Agdia, USA)), y a la qPCR con los cebadores específicos: Cit 295 y Cit 297. Se procesaron y evaluaron 245 muestras foliares de plantas asintomáticas o con síntomas típicos de HLB en lima ácida Tahití, limón pajarito, naranja, *Swinglea glutinosa,* mandarina, *Murraya paniculata,* limón mandarina y pomelo, de predios de Ponedera (Atlántico), Dibulla (La Guajira) y Ciénaga (Magdalena). Todas las muestras se analizaron por inspección visual, yodo macerado y yodo raspado, 98% por LAMP y 53,4% por qPCR. Se encontró 81,6, 68,6, 72,7, 53,3 y 54,3%, de muestras positivas por inspección visual, yodo macerado, yodo raspado, LAMP y qPCR respectivamente, con diferencias significativas en la frecuencia de los resultados entre la inspección visual y las otras técnicas ($P < 0,05$), pero sin diferencias entre el yodo macerado y raspado ($P = 0,41$), ni entre la LAMP y la qPCR ($P = 0,91$). De las muestras asintomáticas, el 20 y 17,8% mostró reacción positiva por yodo macerado y yodo raspado, mientras que el 8,9 y 22,2% fueron positivas a la presencia de CLas por LAMP y qPCR. La técnica de tinción de yodo es una prueba efectiva y económica que ayuda en la confirmación de plantas con síntomas atípicos. La selección de una técnica u otra está relacionada con la disponibilidad de infraestructura, recursos y capacidades técnicas.

Relationship between the high photosynthetic efficiency and leaf anatomic traits in *Solanum pennellii* introgression sublines

Omena-Garcia, Rebeca Patrícia; Martins, Sandy Bastos; **Faria de Souza, Kaík;** de Souza Izabel, Junio; Araújo, Wagner; Nunes-Nesi, Adriano.

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa-MG, Brazil.

Higher efficiency in the photosynthetic process can be achieved by genetic approaches through the identification of genetic variation. By using two *Solanum pennellii* introgression lines (ILs) in a *Solanum lycopersicum* background, we previously demonstrated that IL2-5 exhibits high photosynthetic efficiency associated with altered biochemical components of photosynthesis, such as higher RuBisCO levels under non-photorespiratzory conditions. To understand the physiological, anatomic, and genetic bases of these traits, we characterized plants from introgression Sub-lines (Sub-ILs) of *S. pennellii* (2-5-2, 2-5-6, 2-5-12), IL 2-5, and the parental M82 under ambient and high [CO2], 400 and 800 ppm CO2, respectively. The Sub-ILs 2-5-2 and 2-5-6 exhibited higher photosynthetic capacity compared to M82 in both ambient and high [CO2], accompanied by increases in total biomass production under high [CO2]. These Sub-ILs also exhibited high biochemical capacity (maximum carboxylation velocity by RuBisCO and maximum electron transport rate) under ambient [CO2]. The Sub-IL2-5-12 stood out for exhibiting high water use efficiency under high [CO2], with reduced photosynthesis without changes in stomatal conductance compared to M82. High specific leaf area accompanied by high palisade parenchyma thickness in Sub-IL2-5-12 at high [CO2], opposite results to those observed in Sub-ILs with high photosynthesis. Together, these results suggest that anatomical and photo biochemical components are the bases for the high photosynthetic rates observed in the studied Sub-ILs. Additionally, the three Sub-ILs allowed us to identify a smaller genomic region than that previously identified by the IL2-5. Further *in silico* analysis of the smaller genomic region defined by these Sub-ILs can be further investigated to identify key genes related with photosynthesis in tomato.

Acknowledgments: Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG.

How nocturnal warming could lead to *Deschampsia antarctica* (Poaceae) cold deacclimation?

López, Dariel[1]; Sanhueza, Carolina[2]; Bascuñán, Luisa[2]; Larama, Giovanni[3]; Bravo, León[1].

[1]Laboratorio de Fisiología y Biología Molecular Vegetal, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Forestales & Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile.
[2]Universidad de Concepción, Concepción, Chile.
[3]Centro de Genómica Nutricional Agroacuícola (CGNA), Temuco, Chile.

Asymmetric warming produces a greater increase in minimum nocturnal than in maximum diurnal temperatures; and the phenome in Antarctica could lead to *Deschampsia antarctica* cold deacclimation. We hypothesize that *D. antarctica* freezing tolerance would be reduced mainly by nocturnal temperature increase rather than diurnal; mostly by the downregulation of transcription factors such as CBFs or their target genes and the upregulation of vegetative growth genes. Therefore, our objective was to determine the effect of diurnal and nocturnal warming on cold deacclimation of *D. antarctica* plants, under laboratory conditions. Fully cold-acclimated *D. antarctica* plants (0/8°C) were transferred to 4 treatments for 14 days: one control (0/8°C) and the other 3 with different warming: diurnal (0/14°C), nocturnal (6/8°C) and diurnal-nocturnal (6/14°C) in growth chambers. After 14 days of treatment: freezing tolerance, as lethal temperature to 50% of leaf tissue (LT_{50}), ice nucleation temperature, freezing point and dehydrins levels and total soluble sugars were evaluated. Also, RNA-seq was performed and genes differential expression was bioinformatically analyzed. Nocturnal warming significantly reduced the LT_{50} > 7°C, as well as the ice nucleation temperature and freezing point > 1°C, with respect to the control; as well as dehydrins levels which dropped below the detection limit. While sucrose content was reduced in all warming treatment 38% at least. Nocturnal warming also, significantly reduced the gene expression of CBF-like transcription factors associated with cold stress response, UDP-glycosyltransferase and Sucrose synthase enzymes, Cold regulated (COR) and Dehydrins, among other cold tolerance related genes; compared to cold acclimated and diurnal warming treatments. Consequently, nocturnal warming has a greater effect on cold deacclimation process in *D. antarctica*, than a diurnal temperature increase. This may have important eco-physiological implications because there is evidence of asymmetric warming occurring in nature.

Symposium 2: Biotechnology Applied to Health

Co-chairs: Dr. Pamela Leal Rojas and Dr. Luis Salazar Navarrete

Development of enzymes for viral detection by RT-LAMP.

Brauer-Massirer, Katlin.

Center for Molecular Biology and Genetic Engineering, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil.

During the SARS-CoV-2 pandemics faced in the last two years, one of the major problems for the health systems was the difficulty of testing equally the population around broad areas of some countries. The pandemics scenario raised the awareness of both the public and private sectors to work together in the combat of current outbreaks and in preparation for future viral diseases. For this aim, in 2021 the Center for Medicinal Chemistry (CQMED) at UNICAMP and the Hilab Clinical Laboratory teamed up to improve Hilab's point-of-care testing platform for molecular diagnostics through the development of low-cost national molecular reagents. The method of choice for the application was RT-LAMP (Reverse Transcriptase Loop-mediated Isothermal Amplification). As advantages in relation to RT-qPCR, this approach eliminates the need of a thermocycler, has a reduced number of steps, has decreased sample manipulation and lower cost, while maintaining the accuracy when compared to the gold standard RT-qPCR. Thus, our center defined the objective to obtain nationally produced enzymes applicable for various diagnostics exams.

An innovative multi-omics approach for bioactive metabolites discovery in actinobacteria and cyanobacteria from extreme environments.

Barrientos Díaz, Leticia[1,2,3]; Núñez-Montero, Kattia[4]; Santos, Andrés[5]; Zárate, Ana María[1,3]; Leal, Karla[1,3]; Contreras, María José[1,3]; Salazar, Rodrigo[1,3]; García, Matías[1,3]; Gonzáles, Dayaimi[1,3]; Bruna, Pablo[1,3]; Alarcón, Jonathan[1,3].

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[4]Centro de Investigación en Biotecnología, Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica.
[5]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain.
[5]Departament de Genètica i de Microbiologia, Facultat de Biociéncies, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

Bacteria have been recognized as one of the significant sources of natural products in drug development. However, discovering novel microbial bioactive metabolites is becoming difficult because some bioactive compounds are being rediscovered more frequently. Nevertheless, most of the metabolic diversity of some groups of microorganisms has not yet been revealed, and they still are a promising source of valuable novel metabolites. Bacterial groups such as rare Actinobacteria and Cyanobacteria have been suggested as an untapped source to search for important novel bioactive metabolites, particularly those in highly inhabiting environments. It has been reported that these groups of bacteria can harbour a significant number of biosynthetic gene clusters (BGCs) for the production of multiple secondary metabolites with potential biological activities. Nonetheless, most of the BGCs are cryptic or remain silent under traditional culture conditions. This research aims to ensure that silent novel metabolites from untapped rare Actinobacteria and Cyanobacteria can be activated by accurate elicitation stimuli, which will be a suitable approach for potential drug development against current drug-resistant major health threats. Our current strategy is based on the activation/elicitation of bacterial secondary metabolites through a high throughput (HTP) microbioreactor culture method of rare Actinobacteria and Cyanobacteria strains previously isolated from extreme environments. The activation of novel metabolic pathways will be performed by HTP integrative-omics approach, including metabolomic profiling through LC-QTOF-MS/MS under multiple culture conditions and simulations of environmental threats; and whole genome sequencing followed by genome mining for phylogenetic-guided prioritization of the most divergent BGCs. Strains and metabolic pathways of interest for activation will be selected based on the previous data. This research will provide a robust validation opportunity to boost the potential candidate genes and metabolic pathways for increasing bioactive molecules production of untapped extreme bacteria avoiding the rediscovery of natural products.

New evidence of the interaction between the commd1 protein and the antitumor peptide CIGB-552.

Gómez, Nivaldo; Rodríguez Ulloa, Arielis; González, Luis; Fernández, Julio.

Center for Genetic Engineering and Biotechnology, La Havana, Cuba

CIGB552 is a second-generation synthetic antitumor peptide consisting of twenty amino acids. Its biological target is the pleitropic protein COMMD1 that achieves greater expression in cancer cells. The positive regulation of COMMD1 inhibits the activity of the nuclear transcription factor kappa B facilitating its ubiquitination and subsequent degradation by the cellular proteasome. Define the key regions and amino acids involved in the interaction of protein and peptide. The recombinant protein COMMD1 was expressed in *E. coli* BL21 from a clone obtained from a previous cloning. Purification was done by affinity chromatography. A crosslinking was performed between CIGB552 and COMMD1 using Dimethyl Suberimidate as a crosslinker reagent. Trypsin was used for digestion, and once the peptide fragments were obtained, they were analyzed by LC-MS / MS. 1.5 mL of purified recombinant COMMD1 protein with purity greater than 95% with 4 mg/mL concentration was obtained. Crosslinking was effective for the reaction at room temperature for 1 hour with molar ratio 1:2 between protein and peptide respectively, with a molar excess of 30 times of Dimethyl Suberimidate, the reaction was confirmed by SDS-PAGE and Western Blot against CIGB552. The graphs obtained from LC-MS/MS reveal that the interaction is given between the residues Lys 1, Lys 5, Lys 12, Lys 14, and Lys 18 of CIGB552 and the residues Lys 28, Lys 78, Lys 100 of COMMD1. The results show that CIGB552 interacts with COMMD1 in the region of its N-terminal. This conclusion differs from previous results of in vitro interaction through detection experiments of two yeast hybrids, which propose interaction by the C-Terminal region. Knowledge of the interaction sites will allow the design of a new peptide with greater antitumor activity

Bacteriocins and its potential effect as cell death mediator in colon cancer cells lipid membrane: An *in-silico* analysis.

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Colorectal cancer (CRC) is the third most common cancer worldwide. Once the occurrence of cancer has been diagnosed, chemotherapy is recommended as the main treatment regimen, after surgery. However, although these therapies are relative efficient in initial stages, they produce adverse effects on the patient. Therefore, the search for novel treatments continues. Recent studies on small peptides named bacteriocins, have revealed their capability to modulate cell death in a wide variety of cancer cells. Nevertheless, its full mechanism of action, by which it can generate the effect of cell death, has not yet been identified. Therefore, understanding the interaction of these peptides with their target could guide their use as adjuvant treatment for colorectal cancer patients. First step corresponds to select by inclusion/exclusion parameters from bacteriocins database, selecting sixteen candidates. Structures were obtained from Protein Data Base or by homology modelling by Modeller v10.3. Initial analysis of the sixteen structures were conducted to characterize them. Complex formations were performed between each bacteriocin, and a cancer lipid membrane composed by 50, 34, 16 % of POPC, POPS, & POPE lipids, respectively. Molecular dynamics (MD) was be performed with gromacs software and Martini Coarse-Grain methodologies, leading the analysis of system modification by the insertion of each bacteriocins into its membrane. We characterized bacteriocins models obtained by homologies modelling, leading us to identify relevant effects among the possible interactions of each bacteriocins with the cancer cell membrane. Also, the analysis allowed to evaluate the affinity energy of each complex and thus manage to suggest the best candidate within the 16 bacteriocins, which will be selected for further evaluation. We highlight the effectiveness and possible mechanism of interaction in the complex bacteriocin-lipid membrane. These results comprise the first step of several analysis with the intention of full characterize the interaction and effect of bacteriocins in cellular environments, giving the first perspective of the effect of bacteriocins over cancer cell.

Identification and characterization of proteic interactors for the C-terminal region of SCO-spondin during embryo development.

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The CNS develops from the neural tube, a hallor structure filled with embryonic cerebrospinal fluid (e-CSF) and surrounded by the neuroepithelium, that will generate all the cells of the adult CNS. At the time of maximum proliferation and differentiation of neuroepithelial progenitor cells, the eCSF displays a dynamic expression pattern of essential growth and survival factors, such as fibroblast growth factors, liproteins and SubCommisural Organ spondin (SCO-sp). These molecules must act in a coordinated and interrelated fashion to promote the correct brain development, although the interaction of the eCSF molecules is currently unknown. SCOsp is a huge glycoprotein with multiple matricellular domains capable of interact with other factors of the eCSF, regulating their effects. Our research group recently identified a new isoform of SCOsp composed by the C-terminal region of the full-length protein. The matricellular domains found in this isoform lead us to suggest a modulatory function over neurogenic eCSF factors. The interactors of C-term SCO-sp were analysed by incubation with eCSF, co-immunoprecipitated with anti-SCOsp and MS/MS analyses of the immunoprecipitate. The neurogenic role of C-term SCO-sp was analysed in vitro in a neural precursor cell line and in vivo by electroporation of chick embryos with an expressing vector of C-term SCOsp. Our analysis suggests that C-term SCO-sp interacts with diverse eCSF factors, decreasing their neurogenic effects. The results obtained suggest a relevant role of SCO-sp isoforms in the modulation of eCSF during brain development.

Chromosomal Radiosensitivity in lymphocytes of Cancer Patient Measured After G0 in Vitro Irradiation and G2-Checkpoint Abrogation by Caffeine.

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Individual radiosensitivity is one of the factors associated with adverse reactions to radiotherapy, which sometimes leads to the temporarily or permanently stopping the treatment. The radiosensitivity of human lymphocytes measured using a G0 assay has been linked with an individual's risk of developing normal tissue complications following radiotherapy. Secondly, there are some evidences that cell cycle checkpoint control in stimulated, irradiated lymphocytes may also contribute to the association between chromosomal radiosensitivity and adverse radiotherapy reactions. The identification of radiosensitivity patients before the start of radiotherapy, could favor a modification of the radiotherapy scheme in favor of the quality of life of the patient. Here we present a pilot study to evaluate chromosomal radiosensitivity in lymphocytes of cancer patient after and correlate this finding with adverse reactions after radiotherapy. Twelve patients with head and neck cancer submitted to radiotherapy service, without previous treatment by chemotherapy were evaluated. The blood sample was taken and irradiated in vitro at 6 and 10 Gy before the beginning of the radiotherapy. The G0 caffeine assay was conducted, and the frequency of chromosomal aberrations was evaluated. The oncologist evaluated the adverse effects with a follow up of 2 years. The symptoms were classified in 0-3 grade (CTCAE v4.0), only the symptoms with grade 2-3 were considered. The frequency of chromosomal aberration allowed the radiosensitivity classification of patients in Radioresistant (1/12), Normal (10/12) and Radiosensitive (1/12). Twenty-two symptoms were recorded in all patients. It was obtained an increase of the adverse effects with the increase of radiosensitivity of the patients. The G0 caffeine assay could be used as a test to predict individual radiosensitivity of healthy tissues in patients undergoing to radiotherapy, but more studies with a larger number of patients are needed.

Biotransformation products of drimane sesquiterpenoids with activity against pathogenic yeast.

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Mapuche people and is used in the traditional medicine of this culture. The main secondary metabolites in Canelo are drimane sesquiterpene (SD), among them drimendiol and epidrimendiol showed antifungal activity against pathogenic yeasts of the genus Candida, the main responsible for invasive mycoses worldwide. The prevalence of candidiasis by non-albicans species is increasing, presenting a challenge for the clinical management of this infection due to resistance to azole antifungals. To evaluate the antifungal activity against Candida yeast of drimendiol and epidrimendiol and its biotransformation products by the Antarctic fungus *Cladosporium antarcticum*. C*ladosporium antarcticum* was isolated from sediments isolated from the glacier Collins located in the South Shetland Islands (Antarctic) and identified by sequencing the ITS region of DNA. Drimendiol and epidrimendiol were produced by reduction of polygodial and isotadeonal with NaBH4 respectively. Drimendiol and epidrimendiol were incubated in 500 mL of YM broth inoculated with *Cladosporium antarcticum.* The biotransformation was carried out for 6 days at 15°C, 110 rpm, in darkness. The liquid media was extracted with EtOAc and purified by silica-gel CC. The products were identified by NMR and high-resolution mass spectrometry. The antifungal activity was evaluated by the broth microdilution method against *C. albicans* ATCC90028, *C. krusei* ATCC6258 y *C. parapsilopsis.* The biotransformation of drimendiol produced 3β-hydroxydrimendiol (1) and 9α-hydroxydrimendiol (2), with a yield of 35% and 19.4% respectively. While epidrimendiol produced 9β-hidroxyepidrimendiol (3) with a yield of 44.4%. Product 1 and 2 showed more activity than drimendiol with a MIC of 15 and 25 µg/ml respectively against *C. albicans* and C. *krusei,* and 12.5 µg/ml against *C. parapsilopsis.* While the compound 3 was less active than the starting material epidrimendiol.

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The clinically relevant *Corynebacterium spp* insertion sequences determine their role in resistance and virulence.

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Mobile genetic elements such as plasmids and transposons originate and are transferred as a result of recombinations generated by insertion sequences (IS). Given the threatening emergence of multidrug-resistant opportunistic pathogens, such as some corynebacteria, it becomes necessary to obtain new perspectives on the treatment and prevention of opportunistic nosocomial infections. In this study, we analyze the relationship between insertion sequences, resistance genes, virulence, and the evolution of 11 species of the Corynebacterium genus, which includes species of clinical, veterinary, and biotechnological importance. Among the results, we were able to evidence a common and particular profile of IS among the analyzed *Corynebacterium spp.* We also found that clinically relevant species such as *C.striatum, C. macginleyi,* and *C. diphtheriae* had a higher number of IS per genome. In addition, we found that both resistance and virulence genes are mostly associated with the IS3, IS256, and IS110 families. We also found specific relationships between insertion sequences and virulence genes in *C. diphtheriae,* and the high impact of IS on resistance genes in *C. striatum* was also evidenced. Insertion sequences provide information on the epidemiological status of pathogenic bacteria, including emerging ones, such as corynebacteria. In this study, we establish the first impressions on the distribution of insertion sequence elements according to their environment and epidemiological status in different species of the Corynebacterium genus.

In-vitro anticancerogenic activity preliminary tests for a Costa Rican species of Ganoderma.

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Cancer is one of the leading human diseases, causing 1 in 6 deaths worldwide due to an abnormal or malfunctioning activity of the cell cycle. Researchers are currently looking for ways to confront cancer, and medicinal fungi have gained popularity particularly in Asian countries, since mushrooms have a wide range of applications and have been used historically to treat various diseases, including cancer. In the Ganoderma genus as an example, several bioactive compounds have been studied (including polysaccharides, triterpenoids, sterols, proteins, and peptides) and have been found to show potential in cancer research. Ganoderma lucidum, applanatum, stipitatum, australe, parvulum and amazonense have been found in Costa Rica, as well as a new species of this fungi. Preliminary cell viability tests were conducted on hepatocellular carcinoma (HepG2), osteosarcoma (MG-63) and a normal myoblast cell line (C2C12) using the MTT assay after 24, 48 and 72 hours of exposition to methanolic extract fractions of the new Costa Rican Ganoderma sp. provided by the Biotechnology Research Center from Instituto Tecnológico de Costa Rica. The IC50 after 72 hours was determined for the three cell lines as follows: 0.142 GAE mg/L in hepatocellular carcinoma, 1.100 GAE µg/mL in osteosarcoma and 1.360 GAE µg/mL in myoblasts. This are promising results considering that the fraction of the extract contains in total 10.290 GAE µg/mL. However, it is yet to be determined which component or components from Ganoderma sp. are responsible for the apparent anticarcinogenic activity, as well as a further characterization of the extract and its fractions.

Optomotor response (OMR) more than a visual test. Modeling diseases of national interest.

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The optomotor response (OMR) is a visual-motor response in which zebrafish larvae are stimulated through high-contrast signals. These signals are usually moving-alternating black and white lines that encourage the larvae to swim in the same direction. Our laboratory has shown that modifications to this assay are possible, such as color, speed, and line thickness, achieving greater efficiency. We have shown that the red color evokes a similar response to the classic black, but with a superior contrast between the larva and the line. The OMR is classified as a tool for identification of mutations affecting the visual system. However, our laboratory seeks to give it another application for disease modeling. Metabolic diseases, such as obesity, diabetic retinopathy, and cardiovascular diseases. These diseases are particularly of national interest, Mexico ranks fifth in obesity worldwide and it is estimated that by 2030, 1 in 3 children will suffer from it. Furthermore, the annual increase in childhood obesity is 2.5%. We consider essential to generate tools that allow the study of these diseases. Zebrafish has emerged as a robust new model for various diseases, including cancer, inflammation, and neurodegeneration. It shares multiple advantages with classical models, such as well-established genome editing tools and a fully sequenced genome. Furthermore, zebrafish have 84% of the genes associated with human diseases. We conducted experiments exposing larvae to different conditions to assess learning, heart rate disturbances, and the response of larvae fed different diets. Our experiments demonstrated that larvae exposed to OMR stimuli show obesity-related changes in heart rate and altered response when exposed to toxic substances. Demonstrating that OMR goes beyond a visual test. This test may provide additional tools for modeling metabolic, cardiovascular, and neurodegenerative diseases.

Symposium 3: Cellular and Molecular Biology of Reproduction

Co-chairs: Dr. Pamela Uribe Catalán and Ricardo Felmer Dorner

LncRNAs in the embryo and developmental biology.

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A fully differentiated cell does not, naturally, return to cellular pluripotency. Although, somatic nuclear transfer, cloning, and other methodologies based on exogenous expression, show cellular reversibility through nuclear reprogramming. Embryonic development and cell differentiation originate complex tissues and structures from gene expression. These processes are regulated by a series of complex genetic and epigenetic mechanisms, including, among others, the long non-coding RNAs (lncRNA). LncRNAs are a relatively new class of noncoding RNAs and have recently shown a greater number of genes when compared with mRNAs, an increasing scientific interest, due to their embryonic development and gene regulation role. Furthermore, recently it has been shown that nuclear reprogramming and early embryonic development are regulated by lncRNAs. Using bioinformatics tools, and reverse engineering techniques it is possible to identify and predict lncRNA and their possible involvement in gametes, zygotes, and embryos. Some lncRNAs are somewhat known as the X chromosome inactivation lncRNA XIST, the cell proliferator regulator lncRNA H19, the male fertility essential lncRNA TUG1, and lncRNAs maternally expressed genes (MEG). Although a plethora of new lncRNAs should be discovered in the next years. SIRENA1 is an example of one of those newly discovered lncRNAs, being exclusive to mice, and the most expressed lncRNA in the oocyte is active post-transcriptionally impacting mitochondrial distribution during oocyte maturation. While other lncRNAs that were already known to be involved with disease such as cancer is now finding their importance for development and embryogenesis, like MALAT1 which was known to have involvement with lung cancer and has recently been implicated with trophoblast cell development. Also, most of the little knowledge that we have was mostly discovered in mice and humans, on the other hand, livestock species are economically important and still neglected in this field.

Activation of bovine oocytes by protein synthesis inhibitors: New findings on the role of MPF/MAPKs.

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Oocyte activation by protein synthesis and phosphorylation inhibitors, respectively, have improved the in vitro embryo production rates in different species. This study evaluated the mechanism by which anisomycin (ANY), a protein synthesis inhibitor, activates bovine oocytes alone or combined with cycloheximide (CHX), another protein synthesis inhibitor or dimethyl amino purine (DMAP), a protein phosphorylation inhibitor, on the embryo developmental potential, dynamics of MPF and MAPKs and expression of important embryo quality genes. The results showed that activation of bovine oocytes by ANY, CHX and IVF, inactivates MPF by CDK1-dependent specific phosphorylation without cyclin B1 degradation. CHX or ANY promoted this inactivation, which seemed to be more delayed in the physiological activation (IVF). Both inhibitors modulated MPF via an ERK1/2-independent-pathway, whereas IVF activated bovine oocytes via an ERK1/2-dependent-pathway. Finally, anisomycin does not activate the JNK and P38 kinase pathways. On the other hand, oocyte activation by the dual inhibition of protein synthesis and phosphorylation, induces MPF inactivation without degradation of cyclin B1, while MAPK inactivation occurs differentially between these inhibitors. Thus, although the combined use of these inhibitors does not affect early embryo development, it positively impacts the expression of genes associated with embryo quality.

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Effect of pathological Rho GTPase modulation on function and structure of human spermatozoa.

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Half cases of Infertility are associated to problems in men. Male genital tract infection (MGTI) men have decreased sperm function. Escherichia coli and Chlamydia trachomatis are bacteria that cause MGTI and produce toxins that modulate Rho GTPase somatic cell, altering its structure and function. To date, there are no studies of pathological Rho GTPase modulation on human spermatozoa, so the aim is to evaluate the effect of Rho GTPase modulation on function and structure of human spermatozoa. First, Human spermatozoa were separated in capacitation (CAP) and without capacitation (NOCAP) conditions. Both conditions were exposed to Rho Activator reagent for 2 hours at 37 °C. Later sperm viability, motility, mitochondrial membrane potential (MMP), capacitation, acrosome reaction (AR), calcium content, morphology and RhoA GTPase activity were evaluated. Sperm motility and morphology were decreased in both conditions, but Viability, MMP capacitation were not altered. AR sperm was increased in both condition and Rho Activator concentration. In NOCAP was observed increase of calcium content and RhoA activity. After that, sperm in CAP and NOCAP condition were exposed to Rho Activador and inhibitor reagents for 0, 10 and 120 minutes in order to evaluate sperm motility, AR and RhoA, Rac1 and Cdc42 activity. Rho Inhibitor decrease sperm motility meanwhile Rho Activator only decrease sperm motility at 120 minutes. AR sperm were increased with Rho Inhibitor however Rho activator only do this at 120 minutes in CAP. In NOCAP the effects on AR were observed at 120 minutes. Only RhoA GTPase has activity in spermatozoa at 120 minutes in CAP. All Rho GTPases has activity in NOCAP. Finally, RhoA and Rac1 has activity at 10 minutes in both condition but cdc42 only has activity in NOCAP condition. Pathological Rho GTPase modulation can alter function and morphology of spermatozoa, explaining the way to infection could induce infertility.

Mammalian gametogenesis and fertilisation: Main molecular events in germ cells.

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Animal reproduction in mammals is a complex process involving a variety of factors. Both female and male gametes undergo a series of biochemical and morphological changes to become mature cells and achieve fertilization. These changes include mainly a reduction in the number of chromosomes through meiotic divisions, and morphological changes that allow them to adopt a structure suitable for their functions. During their development, both gametes are supported by different somatic cells, such as granulosa cells, cumulus cells and oviductal epithelium cells in the case of the oocyte, and such as Sertoli cells and cells of the epdidymal epithelium in the case of the spermatozoa. Subsequently, during its passage through the female reproductive tract, the sperm must still undergo a process called "sperm capacitation", which includes a series of molecular cascades that end with tyrosine phosphorylation of various proteins, indicating that the sperm is now capable of fertilizing the oocyte. Upon contact with the oocyte, the spermatozoon releases hydrolytic enzymes present in its acrosome in order to pass through the zona pellucida, a process known as the “acrosomal reaction”. Later, during fertilization the plasma membranes of both gametes fuse and the sperm nucleus is released into the oocyte cytoplasm, as well as other factors necessary for oocyte activation, which is also a complex process involving several molecular signaling pathways. If all these processes occur correctly, a zygote will be formed, which will implant in the endometrium of the mother and will continue its development. In this presentation will discuss the genesis of male and female sex cells, and the main molecular processes they must go through to become mature cells and to form an embryo.

Effects of climate change on sperm function and motility kinematic parameters in sperm from fish.

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Fish mostly are ectotherms and of external reproduction. Water temperature is one of the major environmental cues for fish reproduction. An increasing number of rapid variations in water temperature are being caused by climate change. As a result, whether in a hatchery (artificial fertilization) or in the natural environment, sperm are likely to come into contact with varying activation medium temperatures on their way to an egg. Spermatozoon motility is considered to reflect several metabolic pathways and regulatory mechanisms, any abnormalities of these factors could cause poor sperm motility. The objective of this study was to evaluate the in vitro effect of the temperature of the sperm activation medium in Atlantic salmon (*Salmo salar*) on sperm function and motility. Semen samples obtained by abdominal massage (T1) and testicular dissection (T2) were evaluated after 4 days of storage at 4 °C. Plasma membrane integrity (PMI), mitochondrial membrane potential (MMP), superoxide anion (O2-) production, and DNA integrity were evaluated. The motility kinematic parameters (velocity and progressivity) were evaluated at 8 °C and 16 °C. The IMP remained > 70% during storage in both groups. MMP was higher in the T2 group ($P<0.05$) on days 3 and 4. In addition, cytoplasmic O2- production in T2 was higher on days 2 and 3 ($P<0.05$). However, mitochondrial O2- production was higher in T1 ($P<0.05$), there were no differences in DNA integrity. The motility kinematic parameters were affected by the activation temperature in both groups, 20s post-activation ($P<0.05$). The results indicate the importance of considering the sperm collection method for storage, the temperature of the activation medium when characterizing sperm motility and determining the optimal temperature conditions for in vitro fertilization in *Salmo salar*.

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Neutrophil extracellular traps and their impact on sperm function.

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Human infertility affects 48.5 million couples worldwide, which is approximately between 8 and 12% of reproductive-age couples. In these cases, the male factor represents around 50% of the cases and between 6 and 15% are secondary to urogenital infections. Associated causes include the presence of inflammatory cells with a high production of reactive oxygen species (ROS), cytokines, and neutrophil extracellular traps (NETs), a little studied mechanism in reproduction. The aim of our work has been to characterize the NETs/spermatozoa interaction, focusing on the rate of NETs in sperm function. Initial results showed that the human spermatozoon constitutes a biological stimulus capable of generating the NETosis mechanism *in vitro* and that sperm samples from patients with epididymitis produce more NETs than donors without associated pathologies. In addition, we have demonstrated that NETs reduce progressive motility, damage to the cell membrane and acrosomal damage. Moreover, we found evidence that contact with NETs makes the sperm less able to adhere to the zona pellucida and that this effect can be blocked with different inhibitors of the NETosis process, such as DNAse I and 2APB (intracellular calcium blocker). In conclusion, understanding the induction mechanisms of extracellular networks in the female reproductive tract as well as the urogenital tract could establish therapeutic targets to prevent or inhibit the NETosis, which could contribute to the development of new treatments in human infertility. This contribution to knowledge could mean innovative solutions to improve the diagnosis and treatment of infertility that is among the objectives of the WHO for managing infertility in developed countries.

Effect of sperm treatment with lysolecithin on in vitro outcomes of equine ICSI.

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In vitro production of embryos by intracytoplasmic sperm injection (ICSI) involves the injection of a single spermatozoon into the cytoplasm of a mature oocyte. ICSI in horses has become efficient enough to be considered for clinical and commercial purposes. However, there are substantial differences in the efficiency of embryo production among research groups. Previous studies in other mammalian species have shown that destabilization of the sperm membranes prior to injection improves the competence of ICSI zygotes. Therefore, our aim was to evaluate the sperm treatment with lysolecithin (LL) on developmental potential of equine embryos generated by ICSI. Frozen/thawed sperm from 3 mature stallions were treated for 1 minute with 0.05% of LL. Oocytes were collected from slaughterhouse ovaries by follicular aspiration, from living donors by transvaginal ovum pick-up (OPU) and matured *in vitro* for 24 h. ICSI was performed by the conventional and piezo-assisted methods. Sperm acrosome and plasma membrane integrities were measured by flow cytometry to assess the effects of LL treatment. Heterologous ICSI by using bovine oocytes was performed to assess the ability of the sperm to induce cleavage. The results showed that LL decreased the integrity of the plasma membrane but without affecting the acrosome. Heterologous ICSI indicated that LL-treated sperm lost the ability to activate the oocyte. Finally, the developmental potential and quality of equine embryos produced by ICSI showed no differences after LL treatment, either by using the conventional or the piezo-assisted method. However, because the loss of oocyte-activating capability of the LL-treated sperm, there was no embryonic development without exogen activation. These data suggest that LL treatment in the equine spermatozoon does affect the developmental potential of embryos produced by ICSI.

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Effect of incubation of thawed equine spermatozoa under different capacitating conditions on the sperm viability and membrane fluidity.

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Ejaculated mammalian sperm acquire their ability to fertilize an oocyte during transit through the female reproductive tract, a process known as sperm capacitation. Induction of capacitation in vitro can be achieved by incubating spermatozoa with bicarbonate, calcium, and albumin. However, in the equine species, in vitro sperm capacitation is not efficient. The aim of this study was to evaluate the effect of incubating thawed equine spermatozoa in SP-TALP medium with different capacitation inducers. Spermatozoa were incubated for 120 min at 38.5°C in SP-TALP base medium supplemented with MβCD, IBMX, dbAMPc and the combination of IBMX and dbAMPc. SP-TLP without bicarbonate and albumin was used as a non-capacitating control. Acrosome reaction (PNA-FTC/PI) and membrane fluidity (MC540) were analyzed by flow cytometry. Incubation of spermatozoa in the different capacitating conditions showed no differences in the acrosome reaction, however, greater membrane fluidity was observed in capacitating treatments with capacitation inducers. In conclusion, the addition of capacitation inducers to the SP-TALP medium does not affect the integrity of the plasma membrane, achieving greater membrane fluidity, which is consistent with an increase in the capacitation of equine spermatozoa under these conditions. Future research will focus on analyzing the effect of these treatments on capacitation parameters such as Ca2+, tyrosine phosphorylation and zona pellucida binding capacity.

Isolation of small extracelular vesicles (<200 nm) from bovine follicular fluid by ultracentrifugation.

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Small extracellular vesicles (EVs) are involved in cell-cell communication through the transport of various biomolecules. In the area of animal reproduction, recent studies have demonstrated the implication of EVs in the development of gametes and embryos in different mammalian species; however, very few have proposed the use of EVs as a strategy to improve assisted reproduction techniques, such as *in vitro* embryo production. This work consisted of the isolation and characterization of VEPs from follicular fluid for subsequent use in the in vitro maturation process of bovine oocytes. Isolation of EVs from follicular fluid was performed by differential centrifugation (300 xg, 2,000 xg and 15,000 xg) followed by ultrafiltration (200 nm) and ultracentrifugation (110,000 xg). The EVs obtained were partially characterized by particle size analysis (DLS), electron microscopy and western blot (ALIX, CD9, CD63). The fraction of EVs obtained had a particle size of 191 ± 27.71 nm. The presence of the Evs markers CD9 and CD63 was observed in both the EVs isolate and follicular cell sample, whereas EV marker ALIX was only present in EV isolate samples. These results represent the first step in establishing a protocol for purification of optimal EVs for use in *in vitro* embryo production.

Symposium 4: Opportunities in Research and Applied Science

Co-chairs: Dr. Priscilla Brebi Mieville and Dr. Jorge Farías Avendaño

Innovation and entrepreneurship from the university.

Valdebenito Godoy, Franklin; Sandoval Opazo, Sergio.

Dirección de Innovación y Transferencia Tecnológica, Universidad de La Frontera, Temuco, Chile.

Scientific and Technological Entrepreneurship from Universities is a way of technology transfer that has gained strength in recent times in our country, due to new public policies that promote it and the large amount of advanced postgraduate human capital that exists nationwide. Within this framework, the Department of Innovation and Technology Transfer of UFRO created "Trampolín Lab", which is a program that seeks to promote scientific initiatives with entrepreneurial potential, which are born from research results led by undergraduate and postgraduate students of the UFRO. Under the guidance of “Trampolín Lab”, UFRO has positioned itself, for the last 3 consecutive years, as the number 1 University in awarding ANID VIU projects nationwide, which is a contest that finances results obtained from theses to transform them into a scientific and technological entrepreneurship. The program addresses six work pillars, which are: Intellectual Property and Technology Transfer, Specialized Training, Access to Financing, Team Strengthening, Expert Mentoring, and Internationalization.

New pharmacological formulation for the treatment of gastric cancer: a potential alternative in chemoresistance.

Mora Lagos, Bárbara

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Gastric cancer (GC) is an important cancer-related cause of death worldwide. Cisplatin (CDDP) is the most used drug in the chemotherapy regimen for advanced GC. Unfortunately, the high recurrence rate of GC is attributable to drug resistance. The aim of this study was to evaluate new drug formulations based on the combination of a chemokine receptor antagonist (CRA) and CDDP for use in gastric cancer chemotherapy, which could increase antitumor activity compared to treatment conventional with CDDP alone. AGS R-CDDP cells were previously obtained by a stepwise dosing drug protocol. Cytotoxicity assays were determined by using MTT. Tumoroid formation was performed from AGS R-CDDP cells in low adhesion plates. After 10 days in culture, tumoroid >150 µm in diameter were scored and pharmacological stimuli were added on days 14, 17 and 20. CRA was used alone and in combination with CDDP in all assays. CRA/CDDP combination triggered a re-sensitized on AGS R-CDDP cells, decreasing cell viability, compared to CDDP alone. AGS R-CDDP cells showed a higher potential in the ability to form tumoroids compared to AGS wild type cells. Finally, CRA/CDDP combination inhibits tumoroid formation compared to CDDP alone. Our results indicate that the CRA/CDDP combination sensitized AGS R-CDDP cells and inhibited tumoroid formation in comparison with CDDP alone. This combination could be used as a potential coadjuvant in GC therapy allowing to reduce the doses of CDDP and therefore to reduce side effects.

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Bactomelanin®: Exploiting the potential of bacterial pigments of Antarctic origin as photoprotectors against ultraviolet radiation.

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Microorganisms are considered one of the most promising sources for search and production of bioactive compounds of biotechnological interest. Among them, bacteria offer certain distinctive advantages due to their low sensitivity to environmental changes, their easy scalability, as well as their ability to produce pigments of various shades. Pigments play a key role in molecular processes of all living organisms, as they act as a method of adaptation to various environmental settings and serve a protective function against solar radiation. Phenomena such as the depletion of the ozone layer and climate change contribute to an increase in ultraviolet (UV) radiation at the Earth's surface, generating ocular, skin, and neoplastic diseases. Skin cancer is one of the most common cancers in the world with rates increasing due to intensive exposure to UV rays. Currently, between 2 and 3 million skin cancers are diagnosed worldwide each year. Avoidance of sun exposure and use of high-spectrum sunscreens are among the most effective measures to prevent skin diseases. However, increasing emphasis on daily use of sunscreens raised a number of concerns regarding their safety and toxicity to humans and environment. Through this project, it is proposed to formulate a compound from a pigment with photoprotective properties produced by a microorganism isolated from Antarctic soil. This formulation will be evaluated to measure its physicochemical and photoprotection properties using a biological model simulating its reaction in human skin cell lines against UV radiation. As a result of the project under development, it is expected to obtain a commercial prototype that acts as a barrier against solar radiation, thus becoming a novel biotechnological product, with a wide range of applications and at the same time innocuous for its incorporation in different commercial applications, whether in the dermatological, pharmaceutical, or cosmetic industry.

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Closing Conference

Gestionando tecnologías emergentes: Nuevas herramientas para avanzar desde el laboratorio al mercado.

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La creación de empresas de base científica (start up's) o la incorporación al mundo del trabajo con la industria, pueden ser objetivos evidentes entre quienes se gradúan de programas de doctorado. No solo porque es un requerimiento con el cual la sociedad progresa y enfrenta sus desafíos más apremiantes, sino también por los profundos cambios que está experimentando la academia, con plazas docentes cada vez más limitadas y donde el acceso a recursos pasa por la necesidad de vincularse e impactar en forma más evidente con su entorno. En este marco, pasar del laboratorio al mercado con resultados transferibles, requiere de estrategias sustantivamente distintas de las tradicionales formas de conectar la ciencia con el mercado. Lograr este proceso, donde el riesgo de mercado debe ser eliminado en forma progresiva implica también aprender a desarrollar ciencia al revés, desde el mercado al laboratorio haciendo una correcta combinación de Technology Push con Market Pull.

Posters

Effects of nicotine and the nAChRs antagonist UFR2709 on behavior and gene expression of Sprague Dawley rats.

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Neuronal nicotinic acetylcholine receptors are pentameric transmembrane receptors that belong to the ligand gated ion channel family. Nicotinic acetylcholine receptors are widely distributed through the central nervous system, their main role is to modulate the release of neurotransmitters, namely dopamine, GABA, and glutamate. Due to the important functions nicotinic acetylcholine receptors modulate, these receptors are involved in numerous neurological disorders, such as addiction, anxiety, and major depressive disorder, among others. Considering the above, there is a rising interest for the discovery and development of novel compounds that target nicotinic acetylcholine receptors. The compound UFR2709 is a nicotinic acetylcholine receptors competitive antagonist, previous studies have demonstrated that UFR2709 presents anti-addictive properties when administered to animal models of alcohol intake and nicotine addiction. Having this in mind, there is a need to characterize the effect of UFR2709 on the behavioral response and locomotor activity. The main goal of this research was to study the effect of UFR2709 on a rat animal model. For this purpose, we examined the effect of UFR2709 administration in contrast with nicotine on the Open Field Test and the Elevated Plus Maze Test, we also determined the effect over the gene expression within the striatum. Our results indicate that nicotine administration increased locomotor activity and time spent at the center of the arena. Meanwhile UR2709 had a non-stimulant effect on the animals, similar to the control group. Nicotine induced overexpression of nAChRs subunits in the striatum relative to the control group, UFR2709 diminished the relative expression of some nAChRs subunits (alpha 4, alpha 7 and beta 4) and increased the relative expression of nAChRs subunits alfa 3 and beta 2.

Ejercicio físico e hidrocarburos aromáticos policíclicos presentes en la contaminación del aire en un modelo murino.

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El material particulado presente en la contaminación del aire contiene, entre otros componentes, hidrocarburos aromáticos policíclicos (HAPs). Aunque la exposición a los HAPs se ha convertido en un importante factor de riesgo de enfermedad cardiovascular, los mecanismos por los que estos compuestos contribuyen a aumentar el riesgo cardiovascular no se han explorado por completo. Por otra parte, el ejercicio físico es una estrategia terapéutica en el manejo del riesgo cardiovascular, sin embargo, se desconoce si la práctica de ejercicio físico en contextos de contaminación del aire puede contrarrestar los efectos negativos de la contaminación del aire, particularmente de HAPs. El objetivo de este trabajo fue evaluar los efectos del ejercicio físico sobre marcadores de inflamación, disfunción endotelial y desbalance REDOX, inducidos por exposición a HAPs presentes en la contaminación del aire en un modelo murino. Se planteó un modelo de intervención con ratones macho BALB/c expuestos a una mezcla de HAPs conformada por fenantreno, fluoranteno y pireno con una concentración de 50 µg y ejercicio físico aeróbico, incluyendo un grupo control, un grupo de ejercicio aeróbico, un grupo de exposición a HAPs y un grupo de exposición a HAPs + ejercicio aeróbico. Se determinaron los niveles séricos de citocinas inflamatorias séricas y la expresión génica marcadores de marcadores inflamatorios, disfunción endotelial y desbalance REDOX en el aórtico. Además, se evaluó la expresión de las proteínas ICAM-1 y VCAM-1. Los datos mostraron diferencias significativas en citoquinas inflamatorias, expresión génica y proteica de marcadores de disfunción endotelial en tejido aórtico modulados por el ejercicio físico ($p<0,05$). El ejercicio físico es capaz de modular los efectos negativos de la exposición a HAPs, evaluado a través de marcadores de inflamatorios, disfunción endotelial y desbalance REDOX, en el modelo murino propuesto.

Agradecimientos: FONDECYT N° 1171618 & N° 1171765.

Cytotoxic activity of germacran sesquiterpenes isolated from *Podanthus mitiqui* in prostate cancer cell lines du-145 and 22rv1.

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Prostate cancer (PC) is the second most common cancer in men and the fifth leading cause of cancer death globally. In Chile, PC is the first cancer in incidence and the third in mortality. The 5-year survival rate for localized PC is almost 100%; however, the rate drops to 32% if there is relapse or metastasis. Treatment in advanced stages is based on the drug docetaxel, but resistance to this drug is a clinical problem and due to the limited options of effective chemotherapeutics, the search for new active compounds is imperative. In this regard, Germacrane Sesquiterpene Lactones (GLS) from plants of the *Asteraceae* family, have shown antineoplastic and anti-inflammatory potential with cytotoxic activity against different cancer cells by induction of apoptosis. *Podanthus mitiqui* is a Chilean native shrub that produce GSL, and its metabolites could be an alternative in the search for new active molecules against PC. To determine the cytotoxic activity of Erioflorin, Erioflorin-acetate and Tatridin, isolated from *Podanthus mitiqui* against PC lines 22rv1 and DU-145.MTS assays were used to evaluate cytotoxicity. Data were processed with Graphpad Prism 8.0 software. All assays were verified in technical and biological triplicate. The three GSL displayed activity against PC cells in a dependent concentration, showing that Erioflorin-acetate is the most active compound with an IC50 of 1.68 µM and 1.08 µM against 22rv1 and DU-145 respectively, followed by Erioflorin with 4.11 and 3.85 µM and less active compound Tatridin has an IC50 40.10 and 34.92 µM against the same cell lines. Erioflorin-acetate was the GSL with highest activity evaluated against PC, but deeper studies in vitro and in vivo are necessary to evaluate its clinical application.

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Circular RNAs associated with the expression profile of dysregulated microRNAs in heart failure: *in silico analysis*.

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Cardiovascular diseases are a health problem due to their high mortality rate worldwide. Our country has a similar situation, cardiovascular diseases lead mortality rates (27%). Within these diseases, hypertensive, ischemic and cerebrovascular diseases are dominant in our population, which predispose to the development of heart failure. Studies have reported the role of circular RNAs and microRNAs in a large number of pathologies, and their potential regulatory role on RNA species. This function has been described through three main mechanisms, functioning as a modulator of the action of microRNAs and proteins, as well as modifiers of gene expression. One of the mechanisms refers to the ability of circular RNAs to function as "sponges" for microRNAs, capturing them through MRE sites and reducing their availability, decreasing their action on messenger RNAs. RNAseq data was obtained from public repositories. Data were analyzed using bioinformatics tools for circular RNAs (find_circ, CIRI2, and CIRIQuant), ShortCat for microRNAs, and featureCounts for mRNAs. The differential expression of these RNAs was analyzed using the DESeq2 package. circRNA-microRNA-mRNA interaction was analyzed for complementarity using RNAhybrid, and generating networks with CytoScape. Finally, the data was subjected to an enrichment analysis using enrichR. A total of four circRNAs, 3 microRNAs and fourteen differentially expressed mRNAs were obtained, whose parental genes or target sites were detected in molecular pathways linked to heart failure and associated processes, such as cardiac hypertrophy or tissue remodeling. There is a theoretical relationship between the expression profiles of circRNAs, microRNAs and mRNAs, being the sponge mechanism described the main factor, which could cause changes in the gene expression of cardiac tissues of subjects with heart failure.

Metabarcoding of upper respiratory tract microbiomes associated with SARS-CoV-2.

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The SARS-CoV-2 virus that causes severe acute respiratory syndrome or COVID-19 disease, has caused a pandemic with infections in more than 215 countries and caused the death of more than 6.6 million people. The microbiota of the upper respiratory tract is the second richest in abundance in humans, and it has been described that it may play a fundamental role in modulating immune responses against pathogens and that its composition may be altered in cases of viral infections. To see if the microbiota of the upper respiratory tract in SARS-CoV-2 positive patients is associated with the course of a severe COVID-19 disease, a search was carried out in the NCBI database, finding the publication of Galeeva et al. (2022), in which they make available a metabarcoding dataset of 335 nasopharyngeal swab samples from 4 locations in Russia. Using the metadata, 20 hospitalized patients and 20 outpatients were randomly selected to then perform a bioinformatic analysis through the qiime2/2022.2 tool, obtaining significant differences in Alpha diversity with a value of p=0.00093. When performing statistical comparisons of a single factor, using the taxonomy at the genus level, with the Mann-Whitney/Kruskal-Wallis statistical method, significant differences were observed in 5 genera: Granulicatella with a value of p=0.0015, Atopobium, with a value of p=0.0022, Prevotella with a value of p=0.011 and Anaerosinus with a value of p=0.011. The genus Staphylococcus presents a significantly higher abundance in the ambulatory group, compared to the hospitalized group. With the results obtained, it could be said that there is a relationship between the microbiota and the severity of the disease; however, the number of samples would have to be expanded to make it more representative of the population.

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In silico design of a chimeric humanized L-asparaginase.

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Acute lymphoblastic leukemia (ALL) is the most common cancer among children worldwide, characterized by an overproduction of undifferentiated lymphoblasts in the bone marrow. The treatment of choice for this disease is the enzyme L-asparaginase (ASNase) from bacterial sources. ASNase hydrolyzes circulating L-asparagine in plasma, leading to starvation of leukemic cells. The ASNase formulations of *E. coli* and *E. chrysanthemi* present notorious adverse effects, especially the immunogenicity they generate, which undermines both their effectiveness as drugs and patient safety. In this study, we developed a humanized chimeric enzyme from *E. coli* L-asparaginase, which would reduce the immunological problems associated with current L-asparaginase therapy. For these, the immunogenic epitopes of *E. coli* L-asparaginase (PDB: 3ECA) were determined and replaced with those of the less immunogenic Homo sapiens asparaginase (PDB:4O0H). The structures were modeled using the Pymol software and the chimeric enzyme was modeled using the SWISS MODEL service. A humanized chimeric enzyme with four subunits similar to the template structure was obtained, and the presence of asparaginase enzymatic activity was predicted by protein-ligand docking.

The potential induction of copper nanoparticles on human keratinocyte migration and angiogenesis through secretion of proangiogenic factors.

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Wound healing is a multicellular process in which it is essential to stimulate re-epithelialization to repair damaged tissue. A crucial phase is the proliferation, where angiogenesis is triggered. This process involves migration, proliferation, and differentiation of endothelial cells. This process is tightly regulated by multiple growth factors and cytokines that are released at the wound site. Keratinocytes, important for tissue re-epithelialization, are responsible for the secretion of proangiogenic factors such as VEGF-A, PDGF, TGF-α and TGF-β. Several studies suggest that copper can stimulate the keratinocyte migration and proliferation, but at the same time, it is highly cytotoxic. Therefore, it is important to use the nanotechnology tools to overcome these disadvantages, whereby stabilized copper nanoparticles (NpsCu^0) could be an alternative to reduce cytotoxicity and in turn could allow accelerate the healing process. In this study we evaluated the viability, migration, and release of proangiogenic factors from human keratinocytes (HaCaT) stimulated with 1 µM NpsCu^0 at different times (24, 48 and 72 hours). Viability assays were performed using MTS, migration was determined by *in vitro* wound assay. To evaluate the possible induction of NpsCu^0 on the secretion of proangiogenic factors from keratinocytes, these were stimulated with 1 µM NpsCu^0 for 24 hours, and these factors were visualized with a proteome array. The results showed that concentrations of 1 µM NpsCu0 did not affect viability and promote the migration of keratinocytes *in vitro.* The results showed an increased the factors such as ADAMTS 1, CXCL16, HGF and VEGF in conditioned media in compared to controls. These results agree with the literature indicating that these factors are released from keratinocytes. Our results demonstrate that the stimulation of human keratinocytes with NpsCu^0 induces cell migration and secretion of proangiogenic factors. These results are a starting point to explain, in the future, the mechanism of action of copper on the secretion of these proangiogenic factors.

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Atorvastatin modulates differentially the expression of lncRNAs related to cholesterol homeostasis *in vitro*.

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Atorvastatin is extensively used to treat hypercholesterolemia. However, the wide interindividual variability observed in response to this drug still needs further elucidation. Nowadays, the biology of long non-coding RNAs (lncRNAs) is better understood, and some of these molecules have been related to cholesterol metabolism. Therefore, they could provide additional information on variability in response to statins. The objective of this study was to evaluate the effect of atorvastatin on the expression of six lncRNAs (lncRNA ARSR, LASER, CHROME, lncHR1, RP1-13D10.2 and MANTIS) associated with genes involved in cholesterol metabolism *in vitro.* THP1 cells were cultured, differentiated into macrophages with PMA and treated with different doses of atorvastatin (0, 2.5, 5, 10 y 20 µM) for 24 hours. LncRNAs expression were evaluated by relative quantification by RT-qPCR. All lncRNAs showed significant differences in their expression after treatment in at least one of the tested concentrations ($p<0.05$). These results indicate that atorvastatin modulates differentially the expression of lncRNAs related to cholesterol homeostasis *in vitro,* which suggests that these molecules play a role in the variability of the response to this drug; nevertheless, additional studies are needed to elucidate the involvement of this differential regulation on statin response.

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Expression of human chemokines in cisplatin-resistant gastric cancer cells.

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Gastric cancer (GC) is the third most lethal cancer and the fifth most frequent cancer in the world. In Chile, it is the second most common cancer, with a high mortality rate in the Araucanía region, making it a public health problem. The main obstacles for curing GC are late diagnosis and the development of chemo resistant cells to the widely used drug cisplatin. The mechanism of this resistance has not been fully elucidated. However, it has been associated with the secretion of inflammatory mediators such as cytokines and chemokines in the tumor microenvironment. Therefore, the detection of these soluble inflammatory factors in resistant phenotypes may contribute to understanding the mechanisms underlying cisplatin resistance and to inform potential therapeutic targets to improve treatment response in GC patients. To Identify the expression of human chemokines in sensitive AGS (AGS-WT) and cisplatin-resistant AGS (AGS-RCDDP) gastric cancer cell lines. Thirty-eight human chemokines were evaluated using an antibody microarray from cell culture supernatants of AGS-WT and AGS-RCDDP lines, without exposure to cisplatin. RPMI 1640 medium was used as a control. The chemokine microarray images were analyzed in Image J (NIH). Image analysis showed the presence of 4 chemokines: GRO, GROα, CXCL16, and IL-8. The AGS- WT cells expressed all four detected chemokines, while AGS-RCDDP cells only expressed GRO, GROα, and IL-8. The microarray allowed us to detect that the chemokine CXCL16 was differentially secreted in the cell lines studied, being specific AGS-WT cells. Chemokines secreted by AGS cells show a strong association with pro-inflammatory processes, cell migration, and resistance to apoptosis.

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Potential airborne human pathogens: an undesirable inhabitant in built environments, but no considered in indoor air quality standards.

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Potential airborne human pathogens (PAHP) can be a relevant component in air microbiome present in built environments, but they are scarcely considered in standards of indoor air quality (IAQ) worldwide. Studies have showed that PAHP are able to cause diverse diseases, particularly in immunosuppressed patients at medical centers, industrial and non-industrial environments. Studies have also showed that some microbial groups can be found in a wide variety of built environments, while other groups are restricted or indigenous to the interior environment to which it is confined. Therefore, it has suggested that there is a "characteristic microbiome for each environment", including specific taxa associated with the microbiota of the occupants and/or with the activities that take place in each type of environment. In this context, human beings and ventilation systems have been recognized among the main sources that regulate the emission, composition, and dispersion of the air microbiome, and their associated PAHP, in built environments. Although other factors such as temperature, relative humidity, and particulate matter also play a relevant role in the composition and dispersion of the microbiome in indoor environments. Undoubtedly, the recent technological advances have allowed a better description and understanding of the air microbiome present in built environments by modern techniques; however, its spatiotemporal variations, activity, interactions, and particular epidemiological significance of PAHP are still considered as a "black box". In this sense, the main objective of this review is to revise the current information on the taxonomy, influencing factors, methodologies and biohazard associated with PAHP in medical centers, industrial and non-industrial environments as well as their consideration in IAQ standards. Therefore, here we propose that a better knowledge on air microbiome and their associated PAHP is highly required to be considered in the design and establishment of public health policies, regulations, and standards of IAQ in built environments where millions of human beings coexist with other organisms in our modern society.

Influence of previous infection with adenovirus-36 on obesity and insulin resistance in pediatric population.

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Infection with Adenovirus-36 (Ad-36) has been correlated with increased risk of obesity in various populations. Although the mechanisms that promote obesity are not clear, in-vitro and animal studies indicate that Ad-36 promotes lipid accumulation and adipogenic differentiation. Paradoxically, in humans, it has been observed that individuals who are seropositive for Ad-36 (Ad-36(+)) have lower glucose and insulin levels. It is suggested that Ad-36 increases glucose uptake in peripheral tissues independently of insulin, so Ad-36(+) individuals would have a greater risk of obesity but require less insulin. Evaluate the effect of Ad-36 infection on nutritional status and insulin resistance (IR) in a pediatric population. 208 schoolchildren between 9 and 13 years of age underwent pediatric evaluation. Anthropometry and Tanner stage were recorded, and blood samples were taken for biochemical determinations (lipid profile, glucose, and insulin). Nutritional status was defined by CDC percentile criteria using BMI z-score. IR was defined using two criteria for Chilean pediatric population (proposed by Burrows and Barja). Ad-36 seropositivity was determined by ELISA. No association was observed between the Ad-36(+) group and nutritional status. Insulin levels were significantly lower in the Ad-36(+) group ($p = 0.012$). IR was less frequent in the seropositive group according to the Burrows criterion ($p = 0.030$; OR: 0.125; 95%CI: 0.011-0.755) and according to the Barja criterion, no Ad-36(+) individuals had IR. This relationship was confirmed by multiple logistic regression using age and sex as covariates ($p = 0.028$; OR: 0.141; 95%CI: 0.017-1.18). Conclusions: Our results suggest that although there is no association with nutritional status, previous infection with Ad-36 reduces insulin levels and the risk of IR in the Chilean pediatric population. Further studies are needed to confirm these observations and explore the associated mechanisms.

Effects of medium-frequency neuromuscular electrical stimulation on cytokines that influence skeletal muscle regulation in critically ill patients.

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Neuromuscular electrical stimulation (NMES) represents an effective method to attenuate muscle disuse atrophy in critically ill patients. However, is unclear the effects of NMES on cytokines associated with the skeletal muscle mass regulation. The aim of this study was to determine the effects of Medium-frequency NMES on preservation thickness quadriceps muscle dominant and on cytokines associated with the skeletal muscle mass regulation in critical ill patients. Thirty critical ill patients hospitalized in the intensive care unit (ICU) connected to mechanical ventilator were enrolled to this study. Patients were randomized into two groups: Control Group (CG:n=15; age=56+13; APACHE II (Acute Physiology and Chronic Health Evaluation II) disease-severity-score: 32+3), received a standard physical therapy (SPT) program, 2x/day; and Medium-frequency NMES Group (MFG:n=15; age=56+13; APACHE II disease-severity-score: 32+3), received SPT+NMES at 100 Hz and carrier frequency of 2500 Hz, 2x/day. On admission and awakening in ICU the thickness of the quadriceps muscle was evaluated with ultrasonography. Also, IL-6, IL-10, IL-15 and TNF-a serum levels were evaluated by ELISA. The average thickness of the quadriceps muscle dominant at ICU admission was similar between groups (CG: 27.2±8.1 mm, and MFG: 27.4±8.7 mm). In the awakening in ICU, the CG group suffered a 14,64±0.32% reduction in quadriceps thickness. MFG prevented the atrophy, with increase of 17,6±10.67% (Timexgroup, all P=0.001; all η2 0.354) in muscle thickness. Post intervention increased the concentration of IL-15 by 14.29 ± 8.82% in the MFG from 116.47 ± 8.82 to 133.11 ± 16.49 pg/mL and by 5.03 ± 1. Forty-three percent in the CG from 158.10 ± 24.41 to 166.06 ± 24.06 pg/mL (time effect: P=0.002; η2=0.28), without differences between groups (timexgroup: P=0,24; η2=0.048). No changes over time were observed for all other cytokines. Medium-frequency NMES in critical ill patients attenuates muscle mass loss of the quadriceps and stimulates the production of IL-15 cytokine related to protein synthesis in the of skeletal muscle mass regulation.

Validation of a T-maze for the zebrafish (*Danio rerio*) animal model and memory and learning studies.

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Murine models are usually the most used in studies of all kinds, including those of memory and learning. However, in recent years the use of zebrafish as an alternative model has been gaining momentum, expanding the landscape of pharmacology and behavioral studies by allowing cheaper use and higher throughput in assays. Its use in memory and learning studies has also been corroborated through the application of procedures analogous to those used in rodents or through the development of new specific tests for zebrafish. Our primary research objective is to validate the use of this small fish as an animal model in studies of memory and learning. For this purpose, we used tests such as the T-maze (adaptation of the one used with rodents) and we measured the times used by each fish to reach the reward chamber. The measurements were made without exposing the animals to any drug and exposing them to nicotine (a drug with proven action on memory phenomena). After examining the results obtained, it was observed that the zebrafish is capable of remembering the location of the reward chamber, a response that decreases 24 hours after the first exposure. For its part, the nicotine treatment enhanced the performance of the animals that, even after 48h, reported having memories of the location of the camera. With these results we conclude that the use of zebrafish as an animal model is valid and optimal for our future studies.

Influence of seropositivity against adenovirus 36 on the expression of Non-Coding RNAs involved in the adipogenic process in obese subjects.

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Infectobesity or obesity of infectious origin has stood out in recent years, especially infection by adipogenic virus adenovirus 36 (Ad-36). In humans, previous reports associate Ad-36 with obesity, however, information about the molecular mechanisms involved is scarce, being one of the probable ways of its adipogenic effect the induction of PPARγ. To evaluate the expression of non-coding RNAs related to the adipogenic process and other pathways of interest associated with previous Ad-36 infection in visceral adipose tissue (VAT) samples from obese individuals. Fifty-seven obese individuals [(Ad-36 (+); n= 29), (Ad-36 (-); n= 28)] were selected. The miRNome was evaluated by sequencing 8 Ad-36 (+) and 8 Ad-36 (-). The differential expression of miRNAs, miRNA:mRNA interactions, and biological processes involved were evaluated with bioinformatics tools (BaseSpace, miRpath, Ingenuity Pathway Analysis). The expression of non-coding RNAs candidates to modulate adipogenesis and the mRNA of PPARG were evaluated by RT-qPCR. Fourteen differentially expressed miRNAs, including has-miR-27a, hsa-miR-155 and hsa-miR-18a, predictively interact with genes associated with adipogenesis (PPARG and BMP2). Obese subjects Ad-36 (+) presented a higher expression of PPARG (p= 0.008), also presenting an increased expression of the adipogenic miRNAs has-miR-17 (p=0.028) and has-miR-18a (p= 0.042), as well as lower expression levels of anti-adipogenic has-miR-155 (p =0.031), compared to obese individuals Ad-36 (-). Obese Ad-36 (+) also presented lower expression of the anti-adipogenic lncRNAs GAS5 (p = 0.016) and MEG3 (p = 0.034) vs obese Ad-36 (-). Finally, the anti-adipogenic lncRNA GAS5 is negatively correlated with the expression of PPARG (r= -0.917**; p= 0.01). Previous Ad-36 infection modulates the expression of non-coding RNAs involved in the adipogenic process in TAV, suggesting the participation of epigenetic mechanisms in the long-term maintenance of an adipogenic state favored by Ad-36 infection.

Release of immunomodulatory cytokines from human dental pulp stem cells isolated from healthy and inflamed pulps in presence of Lipopolysaccharides.

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Dental caries is a multifactorial infectious disease, the natural permeability of dentin allows bacteria and endotoxins to reach the pulp tissue where they encounter odontoblasts, mesenchymal stem cells, blood vessels and nerves, triggering an intense inflammatory response. The aim of this study was to assess the release of immunomodulatory cytokines affected by the inflammatory status of the donor tissue and/or the maintenance of an inflammatory environment. DPSCs were isolated from six donors (15-29 y.o); three pulps were from caries-free teeth (hDPSCs) and three were from teeth with irreversible pulpitis (uDPSCs). DPSCs from the healthy and the inflamed tissues were cultured in odontogenic and osteogenic media, with or without lipopolysaccharides (LPS). Supernatants were collected after 24 hours, and 7 days of incubation and IL-6 and IL-10 were quantified by enzyme-linked immunosorbent assays. IL-6 and IL-10 were detected in supernatants of DPSCs isolated from healthy and inflamed pulps cultured with odontogenic and osteogenic media with or without LPS. Concentrations of released IL-6 were significantly higher than IL-10 concentrations. IL-6 release was not affected by the origin of the cells in the absence of LPS. On the contrary, the presence of LPS in the culture of hDPSCs and uDPSCs significantly increased the IL-6 release by day 7. The release of IL-10 was higher in uDPSCs compared to hDPSCs on day 1. The presence of LPS in the culture medium did not affect IL-10 release, which was low or undetectable in every group. The high production of IL-6 and the low production of IL-10 from hDPSCs and uDPSCs in the presence of LPS produce a pro-inflammatory net balance. The inflammatory status of the dental pulp should be considered when the use of DPSCs is intended either for research and/or for application in reparative or regenerative therapies.

Antarctic actinobacteria genomic mining for the identification of biosynthetic gene clusters.

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Actinobacteria are a valuable source of secondary metabolites. Most microbial metabolites are produced through metabolic pathways encoded by biosynthetic gene clusters (BGCs). Therefore, bacteria isolated from Antarctica could allow the discovery of new BGCs with biotechnological potential. This work aimed to identify BGCs in eight Actinobacteria isolated from Antarctic soils. For this purpose, the strains were sequenced on the MinION platform of Oxford Nanopore Technologies. The assemblies were evaluated for completeness and contamination, annotated, and subjected to phylogenetic characterisation. BGCs were identified in each genome using antiSMASH v6.1.1, which were compared for taxonomic clustering. The phylogenetic analysis added to the taxonomic classification obtained using the 16S rRNA gene resulted in seven strains being new species due to their low ANI, AAI and dDDH values. In contrast, one strain corresponds to the species Janibacter terrae. Forty-five BGCs belonging to the sequenced strains were identified, of which three groups showed 100% similarity, four groups showed >=50% genetic similarity, and 38 BGCs showed <50% similarity to other known secondary metabolites. In conclusion, the eight strains presented BGCs whose metabolites could be associated with applications such as antimicrobials, antitumors, cosmetics and others, which positions them as excellent candidates for future applications and biotechnological innovations.

Comparative and functional metagenomics analysis of rhizosphere plant communities from the Atacama Desert and Antarctica.

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Antarctica and the Atacama Desert present a diversity of microorganisms adapted to survive under extreme conditions. It has caused great interest to determine the genetic and molecular mechanisms that allow them to establish themselves in these habitats and to contrast the differences between these heterogeneous climates, which enhances the discovery of new bacteria that have not yet been described. Therefore, this work aims to determine the different structures of the rhizosphere microbiota of *Croton chilensis,* Eulychnia iquiquensis, *Nicotiana solanifolia* (Paposo, Antofagasta region), *Deschampsia antarctica,* and *Colobanthus quitensis* from Antarctica and analyses how bioinformatics tools potential functions of this species. Rhizosphere sampling of each plant in the geographical areas was done in triplicate, followed by DNA extraction and sequencing by 16S rDNA analysis using the 16S SQK-RAB204 kit from Oxford Nanopore Technologies. Taxonomic determination was performed with Silva 138 SSU Software and then classification using Centrifuge Software. Multivariate statistical analyses revealed a significant difference ($p \leq 0.05$) between the diversity of bacterial communities in the rhizosphere of desert plants compared to Antarctica. The distribution at the genus level showed a different pattern between the extreme environments, where the genus others 65%, Haliangium 4.03%, Bryobacter 2.5%, unclassified 2.04 % (closest phylum: *Chitinophageceae*) and MNDI 2.01 were the most abundant in the rhizosphere of all samples analyzed. Also, rhizosphere bacterial communities of Antarctic and desert plants share a total of 781 OTUs, and each plant rhizosphere presents a unique group of taxa. Therefore, this study provides essential information that will allow us to explore the biological impact of rhizosphere microorganisms and their functional mechanisms in the establishment of plants in extreme conditions of the Chilean desert and Antarctic.

Biosynthetic gene clusters with biotechnological potential from genomic analyses of Arthrobacter *psychrochitiniphilus* and *Arthrobacter bussei* isolated from Antarctic rhizospheric soil.

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Actinobacteria from extreme environments such as Antarctica generate new metabolic pathways under stress conditions, allowing the synthesis of new bioactive products with unique properties and structures associated with the presence of biosynthetic gene clusters (BGCs). *Arthrobacter* is a genus belonging to the phylum Actinobacteria. It is characterized by many BGCs in its genome for the biosynthesis of natural products and produces pigments with high antioxidant activity and tolerance to UV irradiation. This study aimed to determine the biotechnological potential of *Arthrobacter psychrochitiniphilus* and *Arthrobacter bussei* isolated from Antarctic rhizospheric soil of *Colobanthus quitensis,* previously identified by 16S rRNA gene sequencing and phylogenetic analysis. Four selective media (M1, R2A, ISP-2 and LB) were used to grow the strains at temperatures of 15, 20 and 25ºC, performing routine morphological characterization. DNA was then extracted, quantified, and integrity assessed. Whole genomes were sequenced using MinION (NanoPore). Optimal culture conditions were at 20°C with ISP-2 medium, showing that both strains are Gram-positive with the presence of bacilli and cocci, with *A. bussei* being pink in colour, indicating pigment production. After de novo assembly of long sequences, plus functional annotation, BGCs from various biosynthetic pathways were found to be associated with potential bioactive compounds using the antiSMASH web tool. Emphasis was placed on the search for gene clusters for the biosynthesis of carotenoids as potent antioxidant, antitumour and UV light resistance agents, which are of great utility in the food and pharmaceutical industry.

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Differential expression of genes encoding ATP-independent holdases chaperones in the chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans* ATCC 23270 under oxidative stress.

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Acidithiobacillus ferrooxidans belong to microbial communities involved in the bioleaching of minerals. It can tolerate high concentrations of metals, osmolarity, and desiccation, which may induce oxidative stress by the increase of Reactive Oxygen Species (ROX). These molecules can damage biomolecules like proteins. Since proteins are the most abundant biomolecules in cells, organisms have developed mechanisms to maintain protein homeostasis (proteostasis). In bacteria, protein (re)folding is accomplished by ATP-dependent chaperone systems; however, under stress conditions, accumulation of damaged proteins and decreasing ATP levels can negatively affect their activity. Therefore, to prevent aggregation and facilitate (re)folding of proteins, cells activate ATP-independent chaperones (holdases). The relevance of this system in acidophilic bacteria is still unknown. We studied the expression of the holdase system in *A. ferrooxidans* ATCC23270 and its intracellular ATP concentration upon exposure to oxidative stress with 1mM hydrogen peroxide for 1h. Transcriptional analysis showed that Hsp20.2, Hsp31, and CnoX resulted up-regulated in cultures exposed to H2O2, meanwhile, RidA.1, RidA.2, SlyD, Lon1, Lon2, Lon3, Hsp33 and Hsp20.1 were down-regulated regarding to the control culture. The hsp20.3 copy gene did not show significant differences in mRNA levels between both conditions. The measurement of intracellular ATP levels showed a significantly lower concentration in cells exposed to stress conditions (67%) versus the control cultures (100%). These results suggest that, in addition to the high redundancy of the proteostasis network, there is also a differentiated pattern of gene expression under stress conditions, which could constitute a highly flexible proteostasis system to face multiple environmental challenges of acidic environments when ATP levels are reduced by the effect of the stress. This work paves the way to understanding the proteostasis systems in extreme acidophilic bacteria.

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Neuroprotective activity of Aristoteline, an alkaloid isolated from *Aristotelia chilensis*.

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In Chile, currently 180,000 people suffer from some neurodegenerative disease, and it is estimated that in 2050, 626,000 people will have Alzheimer's or another dementia. Neurodegenerative diseases show a decreased cerebral blood flow (FSC), causing a lack of oxygenation of tissues, increasing oxidative stress, which contributes to the progression of neuropathologies by the generation of highly reactive molecules that produce mitochondrial and DNA damage. *Aristotelia chilensis* is a native chilean tree widely used in folk medicine. Its leaves produce non-iridoid monoterpene indole alkaloids, among them aristoteline showed different pharmacological properties suggesting neuroprotective effects. To determine the neuroprotective activity of aristoteline, evaluating the vasodilator effect in rat aortic rings and the protective activity against proinflammatory stimuli of hydrogen peroxide (H2O2) in human neuroblastoma SH-SY5Y cells. Aristoteline was purified from *Aristotelia chilensis* leaves and chemically characterized by NMR spectroscopy. The vasodilator effect was evaluated by studying vascular reactivity in rat aortic rings. The effect of aristoteline on the contractile response of phenylephrine was studied by preincubating the rings with aristoteline 10-5 M. In the evaluation of cell protection against H2O2, differentiated SH-SY5Y cells were seeded and cultured for 12 h. The cells were then cultured in serum-free DMEM and pretreated with aristoteline and H2O2 (300 µM) for 24 h. Cell viability was determined by MTS assay. Aristoteline produces relaxation in rat aortic rings with intact, denuded or L-NAME preincubated rings with IC50 4.9x10-5 M; 1.1x10-4 M and 5.8x10-5 M, respectively. Moreover, aristoteline restore the cell viability induced by H2O2 at 50 µM. Aristoteline shows vasodilator effects, suggesting that could increase the FSC, moreover aristoteline reduce the cytotoxicity induced by oxidative stress with H2O2 in a neuronal cell model.

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Evaluation of antibacterial and antifungal activity of Canelo (*Drimys winteri*) seed extract fractions.

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Drug resistance acquired by opportunistic microorganisms makes it necessary to search for new compounds to control them. *Drimys winteri* or Canelo has been used by the traditional Mapuche medicine due to the diversity of medicinal properties such as antifungal, antiparasitic and wound treatment. Canelo seed is currently marketed as "Pimienta Mapuche" which has a spicy and pleasant flavor. Canelo produces sesquiterpene type drimane molecules such as polygodial, which has shown antifungal, pain inhibitory, antiparasitic and pungent properties. To evaluate the antifungal and antibacterial activity of fractions and molecules purified from Canelo seeds. For this, 1,223 Kg of Canelo seeds were macerated with EtOAc producing a crude extract which was fractionated with silica gel to obtain fractions of different chemical composition. The fractions were evaluated against *Candida albicans, C. tropicalis* and *C. krusei* yeasts, and *Staphylococcus aureus* and *Escherichia coli,* by disc diffusion susceptibility testing (100 µg/disc). The compounds of the most active fractions were purified by HPLC, and their structures were determined by high resolution Nuclear Magnetic Resonance (600 MHz). Thirteen fractions were obtained, of which F5-F7 were active against yeasts with inhibition halos between 10-18 mm and against *S. aureus* (8-11 mm), while F9-F11 showed activities against yeasts and *S. aureus* (8-10 mm). From F5-F7, polygodial, epipolygonal and epipolygonal acid drimanes were purified, with average activities of 10 mm. Canelo seed extract shows higher inhibition against Candida-type yeasts than against bacteria. The bioactive compounds correspond to drimane sesquiterpenes functionalized at carbons 11 and 12 of the drimane skeleton.

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Determination of the chemical profile and content of total polyphenols in extracts of *Apeiba tibourbou Aubl.* from Mato Grosso State, Brazil.

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Due to the advancement of science and the search for bioactive compounds, for pharmacological and/or biotechnological applications, as in agriculture, the chemical profile of several plant species has been studied. *Apeiba tibourbou Aubl.* is a non-endemic tree native to Brazil known by the popular names as pau-jangada, pente de macaco, jangadeira, and it is distributed from Mexico to Bolivia areas of Americas. In Brazil it occurs in the biomes: Atlantic Forest, Cerrado, Caatinga and Amazon, and plant extracts are used for ethnopharmacological (antirheumatic, antispasmodic and expectorant activities) and biotechnological applications (as ant repellent) by the traditional communities. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Molecular Spectroscopy in the ultraviolet and visible range (UV-Vis) were techniques selected for characterization of plant organic solvent extracts (hidroethanolic extract (70%, v/v) and with hexane, dichloromethane, chloroform, and ethyl acetate) due some advantages as direct analysis, low instrumentation cost and easy operation, producing valuable results for the selection of extracts with the highest concentration of bioactive compounds for further characterization by high performance liquid chromatography with detection by mass spectrometry (HPLC-MS). The results obtained by ATR-FTIR showed the presence of secondary metabolites such as phenolic compounds, tannins and flavonoids and UV-Vis results used by the application of Folin-Ciocalteu method, after analytical method validation, allowed the quantification of total polyphenols in hydroethanolic, chloroform and ethyl acetate solvent extracts of the plant (leaves and barks), where the barks proved to contain higher amount of total polyphenols than the leaves. In addition, as expected, extracts of polar solvents had a higher contend of polyphenols (expressed as gallic acid and pyrogallol concentration).

Effect of the ethanolic extract of Chilean propolis on senescent rat cartilage.

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As life expectancy increases, finding novel strategies that promote healthy aging becomes relevant. One of the most detrimental outcomes of aging corresponds to osteoarthritis (OA), a disabling, complex and multifactorial joint disease. To date, OA-modifying medications are still lacking, for this reason, searching for different therapeutic alternatives becomes necessary. Propolis is a resinous substance rich in polyphenols with multiple pharmacological properties that have been attributed to their antioxidant and anti-inflammatory capacity, which can reduce chronic inflammation associated with aging. For this reason, the main goal of this study was evaluated to evaluate if the in vivo treatment with ethanolic extract of propolis (EEP) could reverse the possible histological alterations generated in cartilage by aging, promoting the viability of chondrocytes and the maintenance of healthy cartilage. Twenty-four male Sprague Dawley (SD) rats of months old were divided into three groups: SR, senescent rats; SR-EEP, senescent rat treated with EEP and SR-V, senescent rat treated with vehicle. The treatment was carried out through oral gavage for 1 month. When the rats were 25 months old, they were sacrificed. The two knee joints were collected for histological description using toluidine blue staining. The tissues were classified according to the OARSI Score and stereological characteristics of the chondrocytes were determined. The articular cartilage of the SR-EEP group was in the process of repair. The cartilage of the FC, M and TP presented a more organized appearance with an increase in thickness, articular surface, and cationic staining of the matrix. The analysis with OARSI scale showed that there was a reduction in the degree of OA and a significant increase in the density of the chondrocytes compared to the SR and SR-V groups ($p<0.005$). Histological and morpho-quantitative analysis of senescent knee articular cartilage suggests beneficial effects of EEP treatment in vivo, improving the OARSI score and increasing the number of chondrocytes present in the cartilage.

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Spatial monitoring sites of *Vaccinium corymbosum* L. crop in the IX Region Chile.

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Climate change has modified the intensity and distribution of rainfall with longer dry periods during the main growing season of crops, being a major limiting factor for plant productivity and yield. Chile has been considered one of the areas with the greatest drought stress, significantly affecting agriculture. The highbush blueberry (*Vaccinium corymbosum L.*) is an important crop fruit in the South central of Chile; being cultivated between VIII-X Region. This work aimed to perform a spectral characterization through remote sensing of highbush blueberry crops in the IX Region. The data was taken from the ODEPA (CIREM) database. Quadrants of 5 km2 were analyzed using Landsat 7 (NASA) satellite images, making calculations from the combinations of spectral bands, where NDVI, SAVI, and NDWI indices in the years 2001, 2017, and 2021 were determined. Statistical analyzes and indices were performed with the GraphPad 8 and QGis programs. The means of NDVI (Normalized Difference Vegetation Index), SAVI (Soil Adjusted Vegetation Index), and NDWI (Normalized Difference Wetness Index) were compared between crop points and between the different years; the means obtained corroborated that the crop points of the blueberry tend to decrease, reaching the maximum in 2017 with Quepe (0.3462) and lower in 2021 with Francisco Millano (0.2696). NVDI did not present significant differences between the different crop locations ($p<0.05$), but there was a correlation between the different years for the same crop point ($p>0.05$). Therefore, the different localities showed similar behavior, decreasing their vegetation indices in the IX Region, consistent with the decrease in the values reached in 2021 corresponding to climate change and drought stress. This exploratory study complements the characterization and spatial monitoring data by remote sensing and spectral study of blueberry cultivars in the IX region. It is highlighted that remote sensing is a powerful tool for monitoring crops of distinct species.

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Evaluation of the antifungal potential of grape cane and fleshed colored potato extracts against Rhizoctonia solani in Solanum tuberosum crops.

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En Chile, se cultiva alrededor de cincuenta mil hectáreas al año de papa (*Solanum tuberosum*), siendo la región de La Araucanía la principal productora y distribuidora de papa del país. El cultivo de papa es un sistema de producción que utiliza altas cantidades de agroquímicos para el control de plagas y enfermedades. Entre las más comunes se encuentra la infección por *Rhizoctonia solani.* Para prevenir o erradicar esta infección se utilizan fungicidas comerciales, sin embargo, su uso intensivo trae consecuencias adversas, por lo cual, la búsqueda de nuevas fuentes naturales con potencial antifúngico es de gran interés. Por ello, este estudio plantea que extractos de subproductos de *Vitis vinífera* y papa de pulpa coloreada (FCP) tienen actividad antifúngica útil para el control de la enfermedad producida por *R. solani* en cultivos de papa. Se realizó un diseño experimental con montaje de invernadero de plantas inoculadas con *R. solani* y tratadas con extractos de VIDES, FCP y fungicidas comerciales, respectivamente. Se determinó parámetros fotosintéticos, compuestos fenólicos y ácidos orgánicos por HPLC-DAD, actividades antioxidantes enzimáticas y no enzimáticas por métodos espectrofotométricos. FCP alcanzó concentraciones de 141 ± 41 mg kg-1 de ácidos hidroxicinámicos y 718,5 ± 102,7 mg kg-1 flavonoles totales. Por otro lado, a medida que el tiempo de aplicación aumenta, la actividad enzimática de FCP y VIDES disminuye en aproximadamente un 50%. En el caso de los compuestos fenólicos estos disminuyen un 62,09% y 29,9% cuando el tiempo de aplicación del extracto aumenta de 15 a 30 minutos, respectivamente. Esto sugiere que dichos extractos, representan una mejora en las plantas de papa en presencia de *R. solani* en comparación al uso de fungicidas comerciales, además son una fuente óptima para la generación de un potencial biofungicida.

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Antibiotics resistance of the bacterial community in sediments from lake Villarica.

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During the last few years, accumulation of antibiotics in lakes has increased as result of anthropic activity, such as agricultural and aquaculture. In this sense, antibiotics and antibiotic resistance genes are considered as emerging contaminants, where lake sediments are highlighted as a hot spot for horizontal gene transfer and the evolution of antibiotic multi-resistant in bacterial pathogens. In this context, the general objective of this research was to explore the antibiotic-resistant bacterial community in the sediments of Lake Villarrica, which has recently been declared as "saturated zone" by Chilean government. In this study, sediment samples from five sampling points (determined by Regional Office of The Environmental Ministry) were collected and studied. The occurrence of antibiotic resistance genes (ARG) to beta-lactam, tetracycline and amphenicol groups (*bla*-TEM, tetM, and catA1, respectively) in the bacterial community was evaluated by qPCR. In parallel, strains resistant to oxytetracycline, chloramphenicol and amoxicillin were isolated by plating method for sediment samples. In addition, ARG spectra to twelve antibiotics commonly used in clinical and veterinary medicine was also evaluated in isolated strains by diffusion discs technique (Kirby-Bauer). Our results showed a significant resistance to amoxicillin in all sampling points. In correspondence with this, the detection of ARG reflected a greater abundance of the beta-lactam group at all sampling points. In addition, a positive correlation was found between bacterial abundance and ARG concentration in all coastlines. It was possible to isolate thirty-two multiresistant strains that will be promptly 16S rRNA gene identified by sequencing. This study allowed establishing a positive relationship between the presence of antibiotic-resistant bacteria and the areas with higher anthropic activity in the Villarrica Lake basin. This study also constitutes one of the few approaches on the use of bacteriological indicators, such as antibiotic resistance, as potential environmental and public health indicators for Chilean lake ecosystems.

Airborne bacterial community in public waiting rooms of medical centers in Temuco city.

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Indoor aerosols are considered indicators of air pollution and may play a role critical role in public health. Particles of biological origin (bioaerosols) are a relevant component of indoor aerosols, including various microorganisms such as bacteria (named as airborne bacteria). In studies developed in hospital environments, a considerable diversity of potentially pathogenic airborne bacteria (PPAB) has been observed that can be associated with the development of nosocomial infections in patients, so medical centers are considered high risk environments. In this context, public waiting rooms (PWR) represent a particular access and shared point with a high flow of people that could harbor large amounts of bioaerosols, including PPAB. In this study, the main objective was to determine the bacterial composition and quantify the PPAB occurrence in indoor aerosol samples of PWR in two medical centers of Temuco city. For this, aerosol samples were taken during the months of March to June of the year 2021 and 2022, using a Coriolis air sampler. The composition of airborne bacteria was analyzed using high throughput sequencing (HTS) analysis (Illumina technology) using 16S rRNA gene libraries. The presence of relevant PPAB in Chile and resistance genes to betalactam and tetracycline antibiotics (*bla*-TEM and *tet*-M, respectively) was also evaluated and quantified by qPCR. The HTS showed Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes as most abundant phyla, while Moraxellaceae, Flavobacteriaceae and Rhodobacteriaceae were the most representative families. The qPCR did not reveal the presence of PPAB in air samples; however, the presence of the bla-TEM and tet-M genes were detected. A significant increase in the concentration of airborne bacteria in PWR was observed during the period from March to June 2022 in relation to those count obtained in 2021. From this point of view, the low flow of people due to sanitary measures and the strict the sanitation protocols applied during the pandemic could be some of the determinant's factors for which a low bacterial concentration was observed, mainly in the period 2021.