

# **Abstracts of the 1 st International Congress of Biotechnology and Bioengineering- V COPEBIOT 2021**



Editor: Centro Interdisciplinar de  
Investigación Marinha e Ambiental –  
CIIMAR

-Author:

Vitor Vasconcelos

-Co-authors:

Antonio M. Lazarte R.

Ronald Huarachi Olivera



**1 st INTERNATIONAL CONGRESS OF  
BIOTECHNOLOGY AND BIOENGINEERING**

**5 th Peruvian Congress Biotechnology  
and Bioengineering**

August 9-13, 2021, UNESA-Arequipa, Peru

**"Year of the Bicentennial of Perú: 200 years of Independence"**

## **Abstracts of the 1 st International Congress of Biotechnology and Bioengineering-V COPEBIOT 2021**

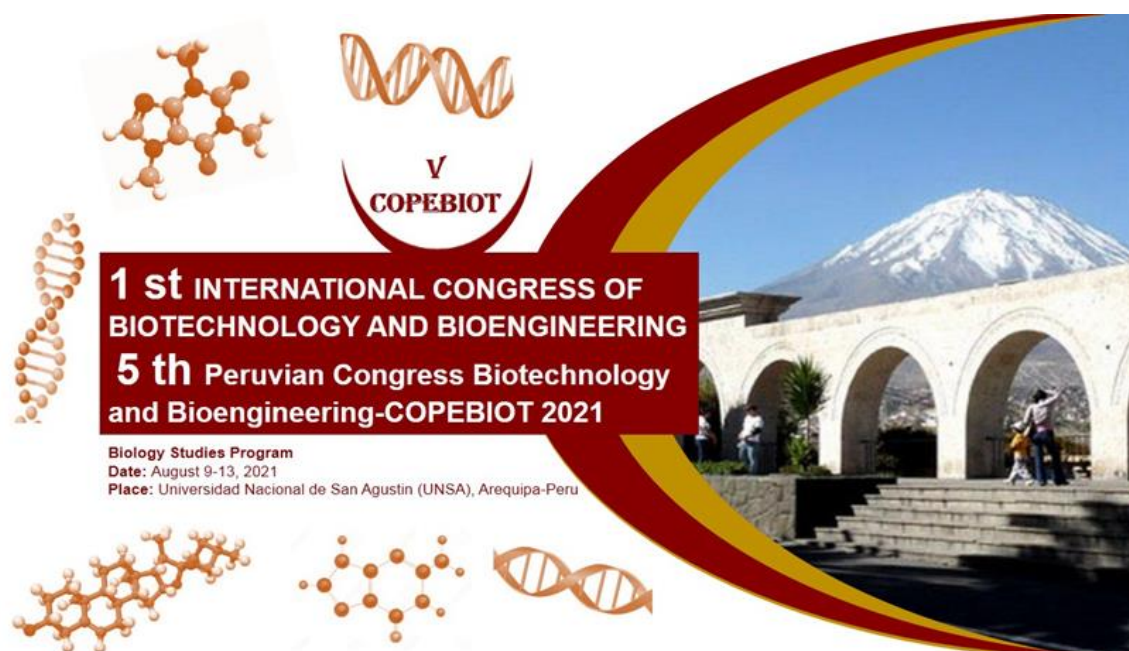
**1 st INTERNATIONAL CONGRESS OF BIOTECHNOLOGY AND BIOENGINEERING**

**5 th Peruvian Congress Biotechnology and Bioengineering-V COPEBIOT 2021**

**Biology Studies Program**

**Date:** August 9-13, 2021

**Place:** Universidad Nacional de San Agustín (UNSA), Arequipa-Perú



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## WELCOME CHAIRS OF THE ORGANIZING COMMITTEE



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Código Renacyt: P0060100



VITOR MANUEL OLIVEIRA VASCONCELOS  
Full Professor - Faculty of Sciences of Porto  
University and Director of CIIMAR

**Dear Colleagues:** It is my great pleasure to thank you for your dedication, participation and great scientific contributions you have accomplished at the 1st INTERNATIONAL CONGRESS OF BIOTECHNOLOGY AND BIOENGINEERING - 5th Peruvian Congress of Biotechnology and Bioengineering-COPEBIOT 2021 which will be held in virtual mode at National University of San Agustín of Arequipa - UNSA. Arequipa, Peru as planned on August 9 to 13, 2021 and will be online allowing speakers and attendees to participate virtually at the same time.

We invite you to submit abstract if you are specialized in fields mentioned below:

- Molecular Biotechnology
- Synthetic Biotechnology
- Medical Biotechnology
- Foods Biotechnology
- Environmental Biotechnology
- Aquatic Biotechnology
- Marine Biotechnology
- Plant Biotechnology
- Food Biotechnology
- Industrial Biotechnology
- Microbial Biotechnology
- Agroindustrial Biotechnology
- Mining Biotechnology
- Biomedical Engineering
- COVID-19 Biotechnology & Research
- Biotechnological perspectives

**Contact:** [vcopebiot.arequipa.peru2021@gmail.com](mailto:vcopebiot.arequipa.peru2021@gmail.com)

**Web:** <https://bit.ly/2SaS2ZF>

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**Design & Scientific Programming:**  
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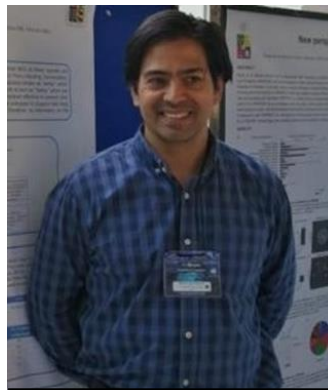
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National University of San Agustín, Arequipa, Perú**

## Speakers at Congress V COPEBIOT 2021

### Plant Biotechnology



PhD. Michael Geoffrey Handford,  
Centro de Biología Molecular  
Vegetal, Facultad de Ciencias,  
Universidad de Chile



PhD. Mario Juan Simirgiotis,  
Universidad Austral de Chile



PhD. Francisca Parada Ramirez  
Centro de Biología Molecular  
Vegetal, Facultad de Ciencias,  
Universidad de Chile



PhD. Laura María Isabel López  
Marchesini  
Universidad Nacional Arturo  
Jauretche & Centro de  
Investigación en Tecnología del  
Cuero (INTI-CIC), Investigador  
Independiente CONICET,  
Argentina



PhD. Natalia Montellano Duran,  
Directora-Ingeniería  
Biotecnológica, Universidad  
Católica Boliviana "San Pablo",  
Bolivia



Pamela Andrea Cabedo Diaz,  
Centro de Biología Molecular  
Vegetal, Facultad de Ciencias,  
Universidad de Chile.



MSc. María Paz Covarrubias  
Becerra, Centro de Biología  
Molecular Vegetal, Facultad de  
Ciencias, Universidad de Chile.

## Aquatic Biotechnology



PhD. Vítor Vasconcelos,  
Interdisciplinary Centre of Marine and  
Environmental Research -  
CIIMAR/CIMAR, University of Porto,  
Department of Biology, Faculty of  
Sciences, Porto University, Porto,  
Portugal



PhD. Ralph Urbatzka,  
CIIMAR, Interdisciplinary Centre of  
Marine and Environmental Research,  
Portugal

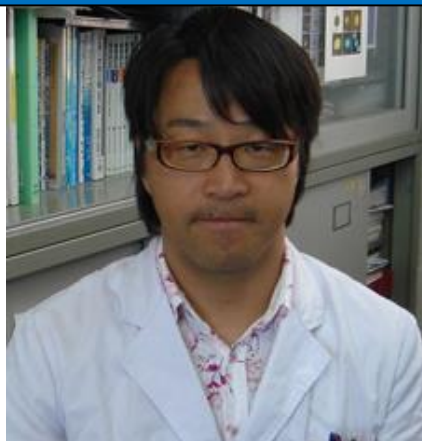


PhD. Sebastián Reyes-Cerpa.  
Centro de Genómica y Bioinformática,  
Escuela de Biotecnología, Facultad de  
Ciencias, Universidad Mayor, Chile



MSc. Carlos Jesus Scotto Espinoza,  
Facultad de Ciencias Naturales y  
Matemática de la Universidad Nacional  
Federico Villarreal, Lima, Perú.

## Marine Biotechnology



PhD. Satoshi Nagai,  
Japan Fisheries Research and Education  
Agency. Fisheries Resources Institute, Japan



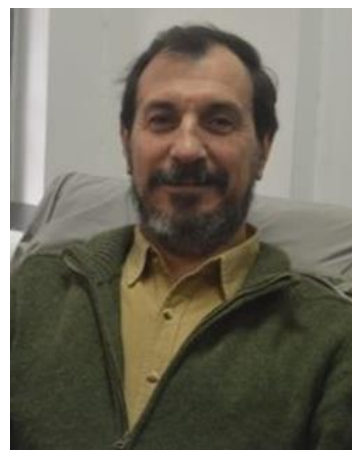
## Environmental Biotechnology



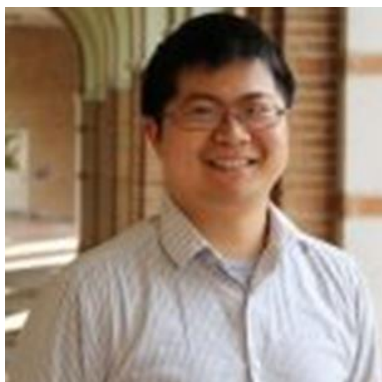
PhD. Claudio Sáez Avaria,  
Centro de Estudios Avanzados,  
Universidad de Playa Ancha, Valparaíso,  
Chile



PhD. Cristina Calheiros,  
Interdisciplinary Centre of Marine and  
Environmental Research  
(CIIMAR/CIMAR), University of Porto,  
Matosinhos, Portugal



PhD. Edgardo Donati  
Centro Científico Tecnológico  
CONICET, Universidad Nacional de  
la Plata, Argentina



PhD. Pingfeng Yu,  
Zhejiang University, People's Republic of  
China



PhD. Armando González Sánchez;  
Instituto de Ingeniería (IIUNAM),  
Universidad Nacional Autónoma de  
México

## Mining Biotechnology



PhD. Mario Esparza Mantilla  
Universidad Nacional de Ingeniería (UNI),  
Lima, Perú



## Industrial Biotechnology



PhD. Jorge Luis Parodi Rivera, Empresa Tonalli Ltda, Chile



PhD. Roberto Jhalver Vega Paulino,  
Universidad Nacional Mayor de San Marcos,  
Lima, Perú.

## Agro-Industrial Biotechnology



PhD. Luiz Pereira da Costa,  
Universidade Federal do Amazonas – UFAM, Brasil

## **Synthetic Biotechnology**



PhD. Cesar de la Fuente, University of Pennsylvania, United States

- **Princess of Girona Foundation Prize for Scientific Research 2021-Spain**
- **Best Young Researcher in USA 2020**

## Medical Biotechnology



PhD. Jorge Alejandro González Cortés,  
Laboratorio de Parasitología Molecular  
Universidad de Antofagasta, Chile



PhD. Ulises Urzúa Tobar,  
Departamento de Oncología Básico-  
Clínica, Universidad de Chile



PhD. Verónica Andrea Burzio  
Menéndez, Universidad Andrés Bello  
Fundación Ciencia & Vida, Chile



PhD. Lucas Daniel Gentilini  
Sartorius, Argentina



PhD. Jorge Bendezu Eguis  
Hasso Group, Biotransfer,  
Universidad Peruana Cayetano Heredia,  
Lima, Perú



PhD. Carla Cynthia Inés Palomino  
Durand,  
CY Cergy Paris University, Francia

## COVID-19 Biotechnology & Research



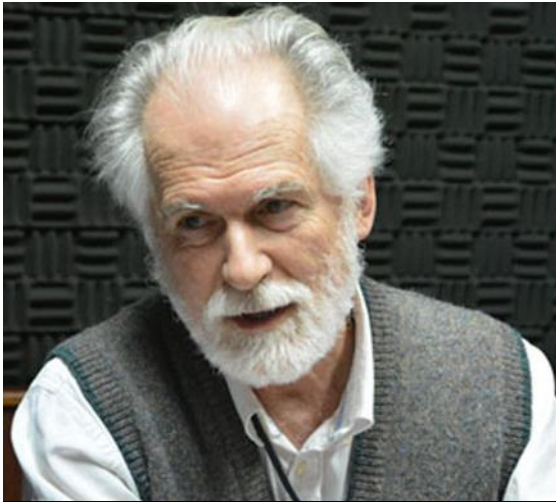
MSc. Henri Bailon Calderón,  
Instituto Nacional del Salud (INS), Perú



Naomi Senehi  
Rice University, United States



## Biomedical Engineering



PhD. Franco Simini  
Universidad de la República, Uruguay



PhD. Luis Vilcahuaman  
Pontifical Catholic University of Peru, Lima, Peru

## Biotechnological perspectives



Dr. Alcibiades Heli Miranda Chavez  
Universidad Catolica de Trujillo, Peru

## Microbial Biotechnology



PhD. Ana Teresa Lombardi,  
Universidade Federal de São Carlos, Botany  
Department, Brasil



PhD. Annette Nicole Trombert Leiva,  
Directora de la Escuela de Biotecnología,  
Universidad Mayor-Chile



PhD. Juan Pablo Cardenas Astudillo,  
Centro de Genómica y Bioinformática, Facultad  
de Ciencias, Universidad Mayor, Chile



PhD. Sandra Figueiredo, CIIMAR  
(Interdisciplinary Centre of Marine and  
Environmental Research), Portugal



PhD. Felix Pompeyo Ferro Mayhua,  
Ministerio de Salud Perú. Región de Salud  
Puno. Red de Salud Chucuito. Universidad  
Católica Portuguesa, Portugal.

## Molecular Biotechnology



PhD. Daniel Leoncio Andrade Sánchez  
University Of Oklahoma, United States



PhD. María Patricia Georgina Sánchez Alonso  
Benemérita Universidad Autónoma de Puebla,  
México



PhD. Gabriel Lassabe Harguindeguy,  
Facultad de Química, Universidad de la  
República, UdelAR, Uruguay



MSc. Ronald Huarachi Olivera  
Centro de Bioinnovación (CBIA),  
Universidad de Antofagasta-Chile



PhD. Maritza Mercedes Calderón Sánchez,  
Universidad Peruana Cayetano Heredia UPCH  
Lima



PhD. Jaeson Calla Choque, University of  
California San Diego (UCSD). United States



# SCIENTIFIC PROGRAMME

## 1 st International Congress of Biotechnology and Bioengineering

### V COPEBIOT 2021

Web: <https://v-congreso-Peruano-de-biotecnologia-y-bioingenieria-2021.webnode.pe/programa/>

Input Link: <https://meet.google.com/jdd-wonf-sin>

Please note that all times are shown as Arequipa, Peruvian (+x) Time. To help you gauge the time difference to your time zone, please refer to the Timezone Converter --> <https://www.worldtimebuddy.com/>

#### Programming of the V COPEBIOT 2021-"Year of the Bicentennial of Peru : 200 years of Independence"

Peruvian time	9 August, Monday	10 August, Tuesday	11 August, Wednesday	12 August, Thursday	13 August, Friday	Peruvian time
8:30-9:15	Opening	Opening Remarks	Opening Remarks	Opening Remarks	Opening Remarks	8:30-9:15
9:15-9:30	Plenary Session	Plenary Session	Plenary Session	Plenary Session	Plenary Session	9:15-9:30
9:30-9:45	Microbial Biotechnology	Environmental Biotechnology	Aquatic Biotechnology	Aquatic Biotechnology	Medical Biotechnology	9:30-9:45
9:45-10:00	PhD. Sandra Figueiredo	PhD. Edgardo Donati	PhD. Ralph Urbatzka	PhD. Vitor Vasconcelos	PhD. Verónica Burzio	9:45-10:00
10:00-10:15	ORAL COMM.-001	ORAL COMM.-017	ORAL COMM.-029	ORAL COMM.-041	ORAL COMM.-052	10:00-10:15
10:15-10:30	ORAL COMM.-002	ORAL COMM.-018	ORAL COMM.-030	ORAL COMM.-042	Masterly Conference(Med. Biot.)	10:15-10:30
10:30-10:45	ORAL COMM.-003	Plenary Session	Plenary Session	ORAL COMM.-043	PhD. Carla Palomino	10:30-10:45
10:45-11:00	ORAL COMM.-004	Medical Biotechnology	Environmental Biotechnology	Masterly conference(Mol. Biot.)	Plenary Session	10:45-11:00
11:00-11:15	ORAL COMM.-005	PhD. Jorge Bendezu Eguis	PhD. Cristina Calheiros	MSc. Ronald H. Olivera	Medical Biotechnology	11:00-11:15
11:15-11:30	ORAL COMM.-006	ORAL COMM.-019	ORAL COMM.-031	Plenary Session	PhD. Lucas Gentilini	11:15-11:30
11:30-11:45	Plenary Session	Plenary Session	Plenary Session	Medical Biotechnology	Plenary Session	11:30-11:45
11:45-12:00	Molecular Biotechnology	Industrial Biotechnology	Environmental Biotechnology	PhD. Ulises Urzua	Biomedical Engineering	11:45-12:00
12:00-12:15	PhD. Gabriel Lassabe	PhD. Jorge Parodi	PhD. Claudio Saez Avaria	ORAL COMM.-044	PhD. Luis Vilcahuaman	12:00-12:15
12:15-14:15	LUNCH	LUNCH	LUNCH	LUNCH	LUNCH	12:15-14:15
14:15-14:30	ORAL COMM.-007	ORAL COMM.-020	ORAL COMM.-032	Plenary Session	Plenary Session	14:15-14:30
14:30-14:45	Plenary Session	ORAL COMM.-021	ORAL COMM.-033	Molecular Biotechnology	Biomedical Engineering	14:30-14:45
14:45-15:00	Microbial Biotechnology	Plenary Session	ORAL COMM.-034	PhD. Daniel Andrade	PhD. Franco Simini	14:45-15:00
15:00-15:15	PhD. Juan Pablo Cardenas	Synthetic Biotechnology	ORAL COMM.-035	ORAL COMM.-045	ORAL COMM.-053	15:00-15:15
15:15-15:30	ORAL COMM.-008	PhD. Cesar de la Fuente	Masterly Conference(Micr. Biot.)	Masterly Conference(Aquat. Biot.)	Masterly Conference(COVID-19 Biot.)	15:15-15:30
15:30-15:45	Plenary Session	Plenary Session	PhD. Felix Ferro	MSc. Carlos Scotto	Naomi Senehi	15:30-15:45
15:45-16:00	Plant Biotechnology	Plant Biotechnology	Plenary Session	Plenary Session	Plenary Session	15:45-16:00
16:00-16:15	PhD. Francisca Parada	PhD. Michael Handford	Agro-Industrial Biotechnology	Aquatic Biotechnology	Molecular Biotechnology	16:00-16:15
16:15-16:30	ORAL COMM.-009	ORAL COMM.-022	PhD. Luiz Pereira da Costa	PhD. Sebastian Reyes	PhD. Maria Sanchez Alonso	16:15-16:30
16:30-16:45	Plenary Session	ORAL COMM.-023	ORAL COMM.-036	Plenary Session	ORAL COMM.-054	16:30-16:45
16:45-17:00	Microbial Biotechnology	ORAL COMM.-024	ORAL COMM.-037	Mining Biotechnology	Plenary Session	16:45-17:00
17:00-17:15	PhD. Annette Trombert	Masterly conference(Plant Biot.)	ORAL COMM.-038	PhD. Mario Esparza Mantilla	Molecular Biotechnology	17:00-17:15
17:15-17:30	ORAL COMM.-010	PhD. Natalia Montellano	ORAL COMM.-039	ORAL COMM.-046	PhD. Jaeson Calla Choque	17:15-17:30
17:30-17:45	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	17:30-17:45
17:45-18:00	ORAL COMM.-011	Plenary Session	Masterly conference(Plant Biot.)	ORAL COMM.-047	Plenary Session	17:45-18:00
18:00-18:15	ORAL COMM.-012	Plant Biotechnology	Pamela A. Cabedo Diaz	ORAL COMM.-048	Medical Biotechnology	18:00-18:15
18:15-18:30	ORAL COMM.-013	PhD. Laura Lopez Marchesini	Masterly conference(Plant Biot.)	Plenary Session	PhD. Jorge Gonzalez	18:15-18:30
18:30-18:45	Plenary Session	ORAL COMM.-025	MSc. Maria Paz Covarrubias	Microbial Biotechnology	ORAL COMM.-055	18:30-18:45
18:45-19:00	Environmental Biotechnology	ORAL COMM.-026	ORAL COMM.-040	PhD. Ana Teresa Lombardi	ORAL COMM.-056	18:45-19:00
19:00-19:15	PhD. Armando Gonzalez Sanchez	Plenary Session	Plenary Session	ORAL COMM.-049	ORAL COMM.-057	19:00-19:15
19:15-19:30	ORAL COMM.-014	Plant Biotechnology	Environmental Biotechnology	ORAL COMM.-050	Plenary Session	19:15-19:30
19:30-19:45	ORAL COMM.-015	PhD. Mario Juan Simiriotis	PhD. Pingfeng Yu	ORAL COMM.-051	COVID-19 Biotechnology & Research	19:30-19:45
19:45-20:00	ORAL COMM.-016	ORAL COMM.-027	Plenary Session	Plenary Session	MSc. Henri Bailon	19:45-20:00
20:00-20:15	Plenary Session	ORAL COMM.-028	Industrial Biotechnology	Molecular Biotechnology	CLOSING	20:00-20:15
20:15-20:30	Marine Biotechnology	Biotechnological Perspectives	PhD. Roberto Vega Paulino	PhD. Maritza Mercedes	CEREMONY	20:15-20:30
20:30-20:45	PhD. Satoshi Nagai	Dr. Heli Miranda Chavez				20:30-20:45

## LIST OF ORAL COMMUNICATIONS

### 1 st International Congress of Biotechnology and Bioengineering V COPEBIOT 2021

NAME	ORAL COMMUNICATION NUMBER	ABSTRACT TITLE	THEMATIC AREA
N. I Valdez	ORAL COMM. -001	Characterization of cyanobacteria <i>Rhabdoderma lineare</i> : a physiological and biochemical approach	Plant Biotechnology
Ana Freire da Silva	ORAL COMM. -002	Microalgae and Cyanobacteria Biostimulant Effects in the Germination of Seeds	Aquatic Biotechnology
Suleivys M. Nuñez	ORAL COMM. -003	Water-Holding Capacity and Antioxidant Activity of Bovine Skin Gelatin Hydrolysates as Potential Polyphosphate Substitutes	Food Biotechnology
Tiago Guimarães	ORAL COMM. -004	Isolation and identification of toxin-producing fungi in macroalgae	Microbial Biotechnology
Diogo A.M. Alexandrino	ORAL COMM. -005	De novo elucidation of the bacterial catabolic pathway of the fluorinated pesticide epoxiconazole	Environmental Biotechnology
Ana Rita Rodrigues Favas	ORAL COMM. -006	The Biotechnological potential of Cyanobacteria and Microalgae Bioactive Compounds in combating the signs of Skin-aging	Environmental Biotechnology
L.A. Quispe-Cueva	ORAL COMM. -007	Comparative study of lymphocyte subpopulations and cytokine profile in blood of patients with COVID-19 and active tuberculosis	COVID-19 Biotechnology & Research
Brando Ortiz-Saavedra	ORAL COMM. -008	Iron supplementation and its effect on the composition of the gut microbiome in 5-month-old infants	Medical Biotechnology
Kenya Arango Farias	ORAL COMM. -009	Determination of bioaccumulation of heavy metals Arsenic (As) and Boron (B) in <i>Arabidopsis thaliana</i> and <i>Chlorella sp.</i> of surface waters of the Rio Tambo, Islay. 2021	Plant Biotechnology
Edgar Chaparro Aguilar	ORAL COMM. -010	Species of <i>Trichoderma</i> producers of $\beta$ -galactosidase in soils of culture of Tacna, Peru	Microbial Biotechnology
Jacqueline Estefany Aza Suaña	ORAL COMM.-011	Bacterial contamination in inert and live surfaces of meat vendors in Puno markets	Microbial Biotechnology
Vannia Alessandra Salas Loayza	ORAL COMM.-012	Antimicrobial effect in transcriptomic studies <i>Tarasa capitata</i> , <i>Tarasa operculata</i> and <i>Tarasa tenuis</i> , from the Arequipa region	Microbial Biotechnology
Carmen Rosario Yauri Chacón	ORAL COMM.-013	Antimicrobial genes in 63 varieties of plants implicated in fungal, viral and bacterial diseases with attenuators of extracts, infusions and essences of plants implicated	Microbial Biotechnology
Cesar Ivan Mejia Llontop	ORAL COMM.-014	Reuse of inoculum from three serial fermentations of ale craft beer on fermentative parameters, volatile compounds and organoleptic properties in final product	Industrial Biotechnology
Cristofer Chambi Mamani	ORAL COMM.-015	Molecular identification and evaluation of resistance to heavy metals by native bacteria isolated from tannery wastewater	Environmental Biotechnology
Rosa Edith Atayupanqui Dueñas	ORAL COMM.-016	Influence of the photoperiod and dilution in industrial wastewater treatment through a microalgae consortium.	Environmental Biotechnology
Ronald Huarachi Olivera	ORAL COMM.-017	Biosorption of Zn (II) from seawater solution by microalgal biomass of <i>Tetraselmis marina</i> AC16-MESO	Environmental Biotechnology
Betsy Rosemary Quispe Cruz	ORAL COMM.-018	Identification and characterization of bacteria with the biodegradation capacity of low-density polyethylene and polyethylene terephthalate isolated from El Tragadero hot spring, Baños del Inca-Cajamarca	Environmental Biotechnology
Ingrid Lucero Cacya Apaza	ORAL COMM.-019	In vitro anticarcinogenic effect of different extracts of microalga <i>Dunaliella salina</i> (Teodoro) on colorectal adenocarcinoma cell line (SW48)	Medical Biotechnology
Eduardo Perochena-Escalante	ORAL COMM.-020	Evaluation of the deproteinization of shrimp waste ( <i>Litopenaeus vannamei</i> ) through the use of proteolytic bacteria and different operating conditions	Environmental Biotechnology
Vanessa Buleje-Alfaro	ORAL COMM.-021	Evaluation of proteolytic activity of bacterial strains isolated from shrimp waste	Microbial Biotechnology
Edgar Lenin Tito	ORAL COMM.-022	Transcriptomic analysis on the influence of the microalgae <i>Chlorella sp.</i> during the germination of <i>Arabidopsis thaliana</i> in water samples from the Tambo River contaminated with As and B	Plant Biotechnology
Anthony Idone Accha	ORAL COMM.-023	Determination of bioaccumulation of heavy metals Arsenic (As) and Boron (B) in <i>Arabidopsis thaliana</i> and <i>Chlorella sp.</i> of surface waters of the Rio Tambo, Islay. 2021	Plant Biotechnology
Edgar Tito Quispe	ORAL COMM.-024	Influence of the extract of <i>Chlorella sp.</i> on the germination and growth of <i>Arabidopsis thaliana</i> under stress by arsenic and boron cultivated with the waters of the Tambo river	Plant Biotechnology
Thiago Lourenço Gomes	ORAL COMM.-025	Obtaining micro and nanocellulose from lignocellulosic biomass from Ingá-Cipó waste ( <i>Inga edulis</i> Mart.) By chemical treatment	Plant Biotechnology
Wilmer Paredes Fernández	ORAL COMM.-026	Molecular characterization of lectins from seeds of <i>Sambucus nigra</i> L. (Elder) and their biotechnological applications	Molecular Biotechnology
Mathias Flores-González	ORAL COMM.-027	Antioxidant activity of the fern <i>Lophosoria quadripinnata</i> (J.F.Gmel) C.Chr.: a potential resource for phytotherapy	Plant Biotechnology
Joseph Obed Ricaldo Sarapura	ORAL COMM.-028	GC-SM of bioactive compounds of <i>Satureja incana</i> 's essential oil	Molecular Biotechnology
Ana C. Fonseca	ORAL COMM.-029	Discovery of novel biological active metabolites from cyanobacteria and actinobacteria: Cytotoxic, anti-obesity and appetite reduction effects	Aquatic Biotechnology
Natália Gonçalves Silva	ORAL COMM.-030	The potential of marine cyanobacteria as a source of lipid-reducing metabolites	Marine Biotechnology
Sandra Pereira	ORAL COMM.-031	Cyanobacterial bioactive nucleosides with antifouling properties	Environmental Biotechnology
Sebastián Caro & Edwin Ccoyllo	ORAL COMM.-032	Biostimulant property assessment of the aqueous extract from invasive alga <i>Caulerpa filiformis</i>	Aquatic Biotechnology
María Elena Suaña Quispe	ORAL COMM.-033	Elaboration of compost from household organic waste and water lentil ( <i>Lemna spp.</i> ) with application of effective microorganisms (EM)	Environmental Biotechnology
Harold Renzo Chirinos Urdy	ORAL COMM.-034	Evaluation of the Physicochemical Properties of Recycled Edible Oils as a source for obtaining Bioglycerin and Biodiesel through Biological Processes, in the city of Arequipa	Environmental Biotechnology
Eliana Mullisaca Contreras	ORAL COMM.-035	Mercury and sediment content of the Azangaro river and its effect on the people of progress	Other Areas not related to Biotech..
Andreza Miranda Barata da Silva	ORAL COMM.-036	Removal of Methylene Blue from Synthetic Wastewater Using Ingá Bark as a Low Cost Biosorbent	Plant Biotechnology



Sidney Souza dos Santos	ORAL COMM.-037	Production of silver nanoparticles mediated by aqueous extracts of tucumã ( <i>Astrocaryum aculeatum</i> )	Plant Biotechnology
Gabriele Simas Ribeiro	ORAL COMM.-038	Optimization of the production of silver nanoparticles mediated by aqueous extracts of açai ( <i>Euterpe oleracea</i> )	Plant Biotechnology
Elizomar Medeiros Barbosa	ORAL COMM.-039	Uranyl salen-type complex as cocatalyst for electrocatalytic oxidation of ethanol	Molecular Biotechnology
Froy Engelbert Coloma Dongo	ORAL COMM.-040	Four micrografting modalities and three auxins (NAA, IAA, IBA) in the in vitro management plants of lemon tree ( <i>Citrus aurantifolia Christm.</i> ) and orange ( <i>Citrus sinensis L.</i> )	Plant Biotechnology
Janaina Morone	ORAL COMM.-041	Natural cosmetics based on cyanobacteria: searching for safe and profitable formulations	Environmental Biotechnology
Diogo Cruz	ORAL COMM.-042	Cyanobacterial Exopolysaccharides from production process to associated bioactivities	Aquatic Biotechnology
Tomé Moreira Azevedo Oliveira	ORAL COMM.-043	Characterization of cyanotoxins and cyanobacteria secondary metabolites from alqueva - first results	Environmental Biotechnology
Liseth Orosco Barrionuevo	ORAL COMM.-044	Contribution of CXCR1 in the generation of enriched sphere cultures in cancer stem cells derived from ovarian cancer cells.	Medical Biotechnology
José González	ORAL COMM.-045	Phenotypic characterization of bacteriophages isolated from wastewater against clinical strains of multiresistant <i>Pseudomonas aeruginosa</i>	Microbial Biotechnology
Jefferson Bustamante Rivera	ORAL COMM.-046	Application of Extremely Acidophilic Microorganisms for the extraction of Cu+2 from Printed Circuit Boards (PCB) by Mycohydrometallurgy.	Mining Biotechnology
Daniela Arequipa & Orquidea M.	ORAL COMM.-047	Copper bioleaching using a native microbial consortium with molecular characterization from ancient mining tailings from the Arequipa region	Mining Biotechnology
Maria Quenta	ORAL COMM.-048	Determination of the transcriptomic response to heavy metals in optional bacteria from the spill of a mining tail in Orcopampa-Castilla, Arequipa, Perú	Mining Biotechnology
Renata Porto Sampaio	ORAL COMM.-049	Growth and biochemical characterization of four Chlorophyta microalgae	Aquatic Biotechnology
Amanda Ellen Lima	ORAL COMM.-050	Effects of differing light colors on the growth and biomolecules in <i>Aphanocapsa holsatica</i> , a Cyanobacteria	Microbial Biotechnology
Lucas Santos Solidade	ORAL COMM.-051	Evaluation of antioxidant activity and polyphenol content of 7 species of microalgae	Plant Biotechnology
Hector Alvarez-Vega	ORAL COMM.-052	Evaluation of the cytotoxicity of chlorophormic extract and chromatographic fractions of <i>Dracontium spruceanum</i> on gastric cancer cells (AGS cell line)	Medical Biotechnology
Dante Rodríguez Alonso	ORAL COMM.-053	Thermographic imaging accuracy in the diagnosis of infected diabetic ulcer in the context of primary healthcare.	Biomedical Engineering
Miluska Vanessa Baylon Cuba	ORAL COMM.-054	Molecular characterization of lactic acid bacteria and protozoa <i>Eimeria sp.</i> , from the gastrointestinal microbiota of <i>Gallus gallus domesticus</i>	Molecular Biotechnology
Eloy Condoni Mamani	ORAL COMM.-055	Cloning, expression and purification of septins and botulinum neurotoxin serotype A (BoNT/A)	Molecular Biotechnology
Brando Ortiz-Saavedra	ORAL COMM.-056	Effect of nafamostat and bromhexine on genes related to viral entry and ACE2 in hACE2 transgenic mice exposed to recombinant spike protein from SARS-COV-2	COVID-19 Biotechnology & Research
Carlos Bailon-Salcedo	ORAL COMM.-057	Analysis of tissue and viral entry proteins in hACE2 transgenic mice exposed to recombinant spike protein treated with nafamostat and bromhexine	COVID-19 Biotechnology & Research



## **LIST OF PLENARY SPEAKERS**

### **1 st International Congress of Biotechnology and Bioengineering** **V COPEBIOT 2021**

NAME	ABSTRACT TITLE	THEMATIC AREA
Vitor Vasconcelos	Cyanobacteria and microalgae collections as sources of valuable bioactive compounds – the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC)	Aquatic Biotechnology
Ralph Urbatzka	Zebrafish as model for marine biodiscovery and metabolic diseases	Aquatic Biotechnology
Sebastián Reyes-Cerpa	Lysosomal reactivation in Atlantic salmon macrophages infected by <i>Piscirickettsia salmonis</i>	Aquatic Biotechnology
Satoshi Nagai	Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital PCR	Marine biotechnology
Cesar de la Fuente	The machine revolution: development of new antibiotics and diagnostic tests	Synthetic biotechnology
Michael Handford	Biotechnological strategies in plants to face the adverse effects of Climate Change	Plant Biotechnology
Mario Simirgiotis	Recent advances in the study of plants in Chile with applications in neurodegenerative diseases	Plant Biotechnology
Laura María Isabel López	Bioprospecting of plant proteases	Plant Biotechnology
Francisca Parada	Increased drought tolerance in tomato plants mediated by changes in the metabolism of brassinosteroids	Plant Biotechnology
Jorge Parodi	A low-cost system for the study of proteins used in salmonid diets, use of proteolysis to determine the quality	Industrial Biotechnology
Roberto Vega Paulino	Effect of reaction conditions on the enzymatic synthesis of isomaltoligosaccharides from forage barley starch ( <i>Hordeum vulgare</i> L.)	Industrial Biotechnology
Edgardo Donati	Microorganisms in the generation of acid drainage. Alternatives for their mitigation	Environmental Biotechnology
Claudio Alejandro Sáez Avaria	Biology of stress in coastal macrophytes and its uses as biomonitoring tools for environmental diagnosis	Environmental Biotechnology
Cristina Sousa Coutinho Calheiros	Nature-based solutions for water management	Environmental Biotechnology
Pingfeng Yu	Enhanced Mutualism Between Soil Phages and Bacteria With Elevated Environmental Stress	Environmental Biotechnology
Armando González Sánchez	Microalgae biotechnology applied to the purification of biogas, combustion gases and the use of residual organic effluents	Environmental Biotechnology
Verónica A. Burzio	Mitochondrial ncRNA targeting induces cell cycle arrest and tumor growth inhibition of MDA-MB-231 breast cancer cells through reduction of key cell cycle progression factors	Medical Biotechnology
Ulises Urzúa	The parity-dependent transcriptome of reproductively aged mouse ovary: prospective markers of ovarian cancer risk	Medical Biotechnology
Jorge González	Role of proteomics in the identification of metabolic pathways associated with virulence of <i>Trypanosoma cruzi</i>	Medical Biotechnology
Lucas Gentilini	mRNA-Based Vaccines: Considerations and Solutions for Successful Manufacturing	Medical Biotechnology
Jorge Bendezu Eguiz	Upstream and downstream processing of cell culture-derived virus particles	Medical Biotechnology
María Patricia Sánchez-Alonso	Behind the secrets hidden at the end of the thread	Molecular Biotechnology
Maritza Calderón	Detection of <i>Toxoplasma gondii</i> by qPCR in clot and cerebrospinal fluid samples from patients with HIV/AIDS	Molecular Biotechnology
Daniel Andrade	HuR Reduces Radiation-Induced DNA Damage by Enhancing Expression of ARID1A	Molecular Biotechnology
Jaeson Calla	The nuclear transcription factor, NR4A3, is required for <i>Plasmodium</i> exoerythrocytic infection	Molecular Biotechnology
Gabriel Lassabe	Development of anti-human IgM small fragment antibodies (nanobodies) as universal reagents for immunodiagnostic of infectious and noninfectious diseases	Molecular Biotechnology
Juan P. Cárdenas	The Human Microbiome: from Evolution to Biotechnological Applications	Microbial Biotechnology
Annette Trombert	Search for new antimicrobial substances or mechanisms against multi-resistant pathogens: the case of <i>Pseudomonas aeruginosa</i> and lactic acid bacteria	Microbial Biotechnology
Sandra A.C. Figueiredo	Exploiting cyanobacterial metabolism for the discovery of new natural products	Microbial Biotechnology
Ana Teresa Lombardi	Increasing biomolecules in microalgae while preserving growth rates. Is it possible?	Microbial Biotechnology
Mario Esparza Mantilla	Mining Biotechnology in the development of antiviral solutions during the Covid19 pandemic	Mining Biotechnology
Luiz Pereira da Costa	Obtaining and Characterizing Cellulose Nanofibers from waste of the orange juice industry using Ionic Liquids	Agro-industrial Biotechnology
Luis Vilcahuaman	Preventive Diagnosis of Diabetic Foot using Smartphone (StandUp H2020 Project)	Biomedical Engineering
Franco Simini	Medicine Based Engineering and Informatics to Foster Patient Physician Relationship	Biomedical Engineering
Henn Bailon Calderon	Development of llama nanobodies against the spike protein of the SARS-CoV-2 virus and neutralization of the virus in cell culture	COVID-19 Biotechnology & Research

**LIST OF INVITED SPEAKERS**  
**1 st International Congress of Biotechnology and Bioengineering**  
**V COPEBIOT 2021**

NAME	ABSTRACT TITLE	THEMATIC AREA
Naomi Senehi	Molecular Imprinting of Coronavirus Attachment Factors to Enhance Water Quality Monitoring and Treatment	COVID-19 Biotechnology & Research
Carla Palomino-Durand	Chitosan-based hydrogels for application in bone tissue engineering	Medical Biotechnology
Carlos Scotto	Gene edition on hydrobiological organisms of aquaculture interest for Peru: Progress and scope	Aquatic Biotechnology
Félix Pompeyo Ferro Mayhua	Ecology and antimicrobial resistance of <i>Ralstonia spp.</i> in the urban water cycle	Microbial Biotechnology
Natalia Montellano Duran	Sensory and microbiological characterization of <i>Allagoptera leucocalyx</i>	Plant Biotechnology
Pamela Cabedo	Studies of tomato lipoyl synthase genes and their potential applications	Plant Biotechnology
María Paz Covarrubias	Functional foods: increased antioxidant capacity in tomato fruits by a higher content of lipoic acid	Plant Biotechnology
Ronald Huarachi Olivera	Circadian transcriptomics in <i>Prorocentrum</i> sp. key dinoflagellate from Algal Blooms of the Eastern South Pacific	Molecular Biotechnology
Heli Miranda Chavez	Perception about genetically modified organisms of the citizens of northern Peru 2021	Biotechnological Perspectives

## **Contents**

### **I. PLENARY SESSIONS**

#### **I.1. Aquatic Biotechnology**

**I.1.1.** Cyanobacteria and microalgae collections as sources of valuable bioactive compounds – the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC).....1

**I.1.2.** Zebrafish as model for marine biodiscovery and metabolic diseases.....2

**I.1.3.** Lysosomal reactivation in Atlantic salmon macrophages infected by *Piscirickettsia salmonis*.....3

#### **I.2. Marine Biotechnology**

**I.2.1.** Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital PCR.....4

#### **I.3. Synthetic Biotechnology**

**I.3.1.** The machine revolution: development of new antibiotics and diagnostic tests.....5

#### **I.4. Plant Biotechnology**

**I.4.1.** Biotechnological strategies in plants to face the adverse effects of Climate Change.....6

**I.4.2.** Recent advances in the study of plants in Chile with applications in neurodegenerative diseases.....7

**I.4.3.** Bioprospecting of plant proteases.....8

**I.4.4.** Increased drought tolerance in tomato plants mediated by changes in the metabolism of brassinosteroids.....9

#### **I.5. Industrial Biotechnology**

**I.5.1.** A low-cost system for the study of proteins used in salmonid diets, use of proteolysis to determine the quality.....10

**I.5.2.** Effect of reaction conditions on the enzymatic synthesis of isomaltooligosaccharides from forage barley starch (*Hordeum vulgare* L.).....11

#### **I.6. Environmental Biotechnology**

**I.6.1.** Microorganisms in the generation of acid drainage. Alternatives for their mitigation.....12

**I.6.2.** Biology of stress in coastal macrophytes and its uses as biomonitoring tools for environmental diagnosis.....13

**I.6.3.** Nature-based solutions for water management.....14



I.6.4. Enhanced Mutualism Between Soil Phages and Bacteria With Elevated Environmental Stress.....	15
--	----

I.6.5. Microalgae biotechnology applied to the purification of biogas, combustion gases and the use of residual organic effluents.....	16
--	----

## **I.7. Medical Biotechnology**

I.7.1. Mitochondrial ncRNA targeting induces cell cycle arrest and tumor growth inhibition of MDA-MB-231 breast cancer cells through reduction of key cell cycle progression factors.....	17
---	----

I.7.2. The parity-dependent transcriptome of reproductively aged mouse ovary: prospective markers of ovarian cancer risk.....	18
---	----

I.7.3. Role of proteomics in the identification of metabolic pathways associated with virulence of <i>Trypanosoma cruzi</i> .....	19
---	----

I.7.4. mRNA-Based Vaccines: Considerations and Solutions for Successful Manufacturing.....	20
--	----

I.7.5. Upstream and downstream processing of cell culture-derived virus particles.....	21
--	----

## **I.8. Molecular Biotechnology**

I.8.1. Behind the secrets hidden at the end of the thread.....	22
--	----

I.8.2. Detection of <i>Toxoplasma gondii</i> by qPCR in clot and cerebrospinal fluid samples from patients with HIV/AIDS.....	23
---	----

I.8.3. HuR Reduces Radiation-Induced DNA Damage by Enhancing Expression of ARID1A.....	24
--	----

I.8.4. The nuclear transcription factor, NR4A3, is required for Plasmodium exoerythrocytic infection.....	25
---	----

I.8.5. Development of anti-human IgM small fragment antibodies (nanobodies) as universal reagents for immunodiagnostic of infectious and noninfectious diseases.....	26
--	----

## **I.9. Microbial Biotechnology**

I.9.1. The Human Microbiome: from Evolution to Biotechnological Applications.....	27
---	----

I.9.2. Search for new antimicrobial substances or mechanisms against multi-resistant pathogens: the case of <i>Pseudomonas aeruginosa</i> and lactic acid bacteria.....	28
---	----

I.9.3. Exploiting cyanobacterial metabolism for the discovery of new natural products.....	29
--	----

I.9.4. Increasing biomolecules in microalgae while preserving growth rates. Is it possible?.....	30
--	----

## **I.10. Mining Biotechnology**

**I.10.1.** Mining Biotechnology in the development of antiviral solutions during the Covid19 pandemic.....31

## **I.11. Agro-industrial Biotechnology**

**I.11.1.** Obtaining and Characterizing Cellulose Nanofibers from waste of the orange juice industry using Ionic Liquids.....32

## **I.12. Biomedical Engineering**

**I.12.1.** Preventive Diagnosis of Diabetic Foot using Smartphone (StandUp H2020 Project).....33

**I.12.2.** Medicine Based Engineering and Informatics to Foster Patient Physician Relationship.....34

## **I.13. COVID-19 Biotechnology & Research**

**I.13.1.** Development of llama nanobodies against the spike protein of the SARS-CoV-2 virus and neutralization of the virus in cell culture.....35

## **II. MASTERLY CONFERENCE**

### **II.1. COVID-19 Biotechnology & Research**

**II.1.1.** Molecular Imprinting of Coronavirus Attachment Factors to Enhance Water Quality Monitoring and Treatment.....36

### **II.2. Medical Biotechnology**

**II.2.1.** Chitosan-based hydrogels for application in bone tissue engineering.....37

### **II.3. Aquatic Biotechnology**

**II.3.1.** Gene edition on hydrobiological organisms of aquaculture interest for Perú: Progress and scope.....38

### **II.4. Microbial Biotechnology**

**II.4.1.** Ecology and antimicrobial resistance of *Ralstonia spp.* in the urban water cycle.....39

### **II.5. Plant Biotechnology**

**II.5.1.** Sensory and microbiological characterization of *Allagoptera leucocalyx*.....40

**II.5.2.** Studies of tomato lipoyl synthase genes and their potential applications.....41

**II.5.3.** Functional foods: increased antioxidant capacity in tomato fruits by a higher content of lipoic acid.....42

### **II.6. Molecular Biotechnology**

**II.6.1.** Circadian transcriptomics in *Prorocentrum sp.* key dinoflagellate from Algal Blooms of the Eastern South Pacific.....43

### **II.7. Biotechnological Perspectives**

**I.7.1.** Perception about genetically modified organisms of the citizens of northern Perú 2021.....44

### **III. ORAL COMMUNICATION**

#### **III.1. Food Biotechnology**

**III.1.2.** Water-Holding Capacity and Antioxidant Activity of Bovine Skin Gelatin Hydrolysates as Potential Polyphosphate Substitutes.....45

#### **III.2. Environmental Biotechnology**

**III.2.1.** Evaluation of the Physicochemical Properties of Recycled Edible Oils as a source for obtaining Bioglycerin and Biodiesel through Biological Processes, in the city of Arequipa.....46

**III.2.2.** Elaboration of compost from household organic waste and water lentil (*Lemna spp.*) with application of effective microorganisms (EM).....47

**III.2.3.** De novo elucidation of the bacterial catabolic pathway of the fluorinated pesticide epoxiconazole.....48

**III.2.4.** The Biotechnological potential of Cyanobacteria and Microalgae Bioactive Compounds in combating the signs of Skin-aging.....49

**III.2.5.** Evaluation of the deproteinization of shrimp waste (*Litopenaeus vannamei*) through the use of proteolytic bacteria and different operating conditions.....50

**III.2.6.** Natural cosmetics based on cyanobacteria: searching for safe and profitable formulations.....51

**III.2.7.** Biosorption of Zn(II) from seawater solution by microalgal biomass of *Tetraselmis marina* AC16-MESO.....52

**III.2.8.** Characterization of cyanotoxins and cyanobacteria secondary metabolites from alqueva - first results.....53

**III.2.9.** Identification and characterization of bacteria with the biodegradation capacity of low-density polyethylene and polyethylene terephthalate isolated from El Tragadero hot spring, Baños del Inca-Cajamarca. ....54

**III.2.10.** Cyanobacterial bioactive nucleosides with antifouling properties.....55

**III.2.11.** Influence of the photoperiod and dilution in industrial wastewater treatment through a microalgae consortium.....56

**III.2.12.** Molecular identification and evaluation of resistance to heavy metals by native bacteria isolated from tannery wastewater.....57

#### **III.3. Industrial Biotechnology**

**III.3.1.** Reuse of inoculum from three serial fermentations of ale craft beer on fermentative parameters, volatile compounds and organoleptic properties in final product.....58



### **III.4. Mining Biotechnology**

- III.4.1.** Copper bioleaching using a native microbial consortium with molecular characterization from ancient mining tailings from the Arequipa región.....59
- III.4.2.** Application of Extremely Acidophilic Microorganisms for the extraction of Cu<sup>+2</sup> from Printed Circuit Boards (PCB) by Mycohydrometallurgy.....60
- III.4.3.** Determination of the transcriptomic response to heavy metals in optional bacteria from the spill of a mining tailer in Orcopampa-Castilla, Arequipa, Perú.....61

### **III.5. Microbial Biotechnology**

- III.5.1.** Bacterial contamination in inert and live surfaces of meat vendors in Puno markets.....62
- III.5.2.** Species of *Trichoderma* producers of  $\beta$ -galactosidase in soils of culture of Tacna, Perú.....63
- III.5.3.** Isolation and identification of toxin-producing fungi in macroalgae.....64
- III.5.4.** Evaluation of proteolytic activity of bacterial strains isolated from shrimp waste.....65
- III.5.5.** Effects of differing light colors on the growth and biomolecules in *Aphanocapsa holsatica*, a Cyanobacteria.....66
- III.5.6.** Characterization of cyanobacteria *Rhabdoderma lineare*: a physiological and biochemical approach.....67
- III.5.7.** Phenotypic characterization of bacteriophages isolated from wastewater against clinical strains of multiresistant *Pseudomonas aeruginosa*.....68
- III.5.8.** Antimicrobial effect in transcriptomic studies *Tarasa capitata*, *Tarasa operculata* and *Tarasa tenuis*, from the Arequipa region.....69
- III.5.9.** Antimicrobial genes in 63 varieties of plants implicated in fungal, viral and bacterial diseases with attenuators of extracts, infusions and essences of plants implicated.....70

### **III.6. Aquatic Biotechnology**

- III.6.1.** Biostimulant property assessment of the aqueous extract from invasive alga *Caulerpa filiformis*.....71
- III.6.2.** Cyanobacterial Exopolysaccharides from production process to associated bioactivities.....72
- III.6.3.** Microalgae and Cyanobacteria Biostimulant Effects in the Germination of Seeds.....73
- III.6.4.** Discovery of novel biological active metabolites from cyanobacteria and actinobacteria: Cytotoxic, anti-obesity and appetite reduction effects.....74

III.6.5. Growth and biochemical characterization of four Chlorophyta microalgae.....	75
--	----

### III.7. Marine Biotechnology

III.7.1. The potential of marine cyanobacteria as a source of lipid-reducing metabolites.....	76
---	----

### III.8. Plant Biotechnology

III.8.1. Optimization of the production of silver nanoparticles mediated by aqueous extracts of açai ( <i>Euterpe oleracea</i> ) .....	77
--	----

III.8.2. Production of silver nanoparticles mediated by aqueous extracts of tucumã ( <i>Astrocaryum aculeatum</i> ) .....	78
---	----

III.8.3. Removal of Methylene Blue from Synthetic Wastewater Using Ingá Bark as a Low Cost Biosorbent.....	79
--	----

III.8.4. Obtaining micro and nanocellulose from lignocellulosic biomass from Ingá-Cipó waste ( <i>Inga edulis</i> Mart.) by chemical treatment.....	80
---	----

III.8.5. Antioxidant activity of the fern <i>Lophosoria quadripinnata</i> (J.F.Gmel) C.Chr.: a potencial resource for phytotherapy.....	81
---	----

III.8.6. Transcriptomic analysis on the influence of the microalgae <i>Chlorella</i> sp. during the germination of <i>Arabidopsis thaliana</i> in water samples from the Tambo River contaminated with As and B.....	82
--	----

III.8.7. Determination of bioaccumulation of heavy metals Arsenic (As) and Boron (B) in <i>Arabidopsis thaliana</i> and <i>Chlorella</i> sp. of surface waters of the Rio Tambo, Islay. 2021.....	83
---	----

III.8.8. Influence of the extract of <i>Chlorella</i> sp. on the germination and growth of <i>Arabidopsis thaliana</i> under stress by arsenic and boron cultivated with the waters of the Tambo river.....	84
---	----

III.8.9. Determination of photosynthetic efficiency in the influence of the microalgae <i>Chlorella</i> sp. during the germination of <i>Arabidopsis thaliana</i> in water samples from the Tambo River contaminated with As and B.....	85
---	----

III.8.10. Four micrografting modalities and three auxins (NAA, IAA, IBA) in the in vitro management plants of lemon tree ( <i>Citrus aurantifolia</i> Christm.) and orange ( <i>Citrus sinensis</i> L.) .....	86
---	----

III.8.11. Evaluation of antioxidant activity and polyphenol content of 7 species of microalgae.....	87
---	----

### III.9. Medical Biotechnology

III.9.1. In vitro anticancerigenous effect of different extracts of microalga <i>Dunaliella salina</i> (Teodoresco) on colorectal adenocarcinoma cell line (SW48) .....	88
---	----

III.9.2. Evaluation of the cytotoxicity of chlorophormic extract and chromatographic fractions of <i>Dracontium spruceanum</i> on gastric cancer cells (AGS cell line) .....	89
III.9.3. Iron supplementation and its effect on the composition of the gut microbiome in 5-month-old infants. ....	90
III.9.4. Contribution of CXCR1 in the generation of enriched sphere cultures in cancer stem cells derived from ovarian cancer cells. ....	91
<b>III.10. Biomedical Engineering</b>	
III.10.1. Thermographic imaging accuracy in the diagnosis of infected diabetic ulcer in the context of primary healthcare. ....	92
<b>III.11. COVID-19 Biotechnology &amp; Research</b>	
III.11.1. Comparative study of lymphocyte subpopulations and cytokine profile in blood of patients with COVID-19 and active tuberculosis. ....	93
III.11.2. Effect of nafamostat and bromhexine on genes related to viral entry and ACE2 in hACE2 transgenic mice exposed to recombinant spike protein from SARS-COV-2.....	94
III.11.3. Analysis of tissue and viral entry proteins in hACE2 transgenic mice exposed to recombinant spike protein treated with nafamostat and bromhexine.....	95
<b>III.12. Molecular Biotechnology</b>	
III.12.1. Molecular characterization of lactic acid bacteria and protozoa <i>Eimeria</i> sp., from the gastrointestinal microbiota of <i>Gallus gallus</i> domesticus.....	96
III.12.2. Uranyl salen-type complex as cocatalyst for electrocatalytic oxidation of ethanol.....	97
III.12.3. Cloning, expression and purification of septins and botulinum neurotoxin serotype A (BoNT/A) .....	98
III.12.4. GC-SM of bioactive compounds of <i>Satureja incana</i> ’s essential oil.....	99
III.12.5. Molecular characterization of lectins from seeds of <i>Sambucus nigra</i> L. (Elder) and their biotechnological applications.....	100
<b>III.13. Other Areas not related to Biotechnology</b>	
III.13.1. Mercury and sediment content of the Azangaro river and its effect on the people of progress.....	101
<b>Index by authors</b> .....	102
<b>ACKNOWLEDGEMENTS</b> .....	113



**I.1.1. Cyanobacteria and microalgae collections as sources of valuable bioactive compounds – the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC)**

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**ABSTRACT**

Cyanobacteria are very diverse organisms in terms of morphology, habitat and ecology and are well known for the diversity of secondary metabolites that they produce either when living isolated or in symbiosis. Among those metabolites, toxins are extensively studied due to the harmful effects they cause on the ecosystems and on human health. Cyanotoxins can have neurotoxic, hepatotoxic, cytotoxic and dermatotoxic properties, being exposure to humans via drinking water, dermal contact during recreation or via food contaminated with the toxins. Apart from producing toxins, and due to their ancestral origin, ecological and biochemical diversity, cyanobacteria are a prolific source of compounds with potential biotechnological applications, namely in the pharmacological field. A wide range of secondary metabolites exhibiting pharmaceutical properties such as antibacterial, antiviral, antifungal, anti-inflammatory and anticancer have been described. Bioactive compounds from cyanobacteria may also have allelopathic activity with potential use to control algal blooms or as antifouling in the marine environment. Cyanobacteria extracts can also prevent the development of some invertebrates and so they can be candidates to develop antifouling agents that are environmentally friendly. The potential of cyanobacteria as source of new bioactive compounds is enormous, with the advantage of being applicable in many different areas of biotechnology, with many industrial applications. To achieve that, culture collections are essential infrastructures as the base for the discovery of new compounds. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource centre located at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), comprising more than 1000 different cyanobacterial and microalgae strains. LEGE-CC strains were mainly isolated from Portuguese ecosystems (including Madeira and Açores Islands) but also from other countries worldwide (e.g. Australia, Brazil, Colombia, Morocco, Mexico, Dominican Republic, Cabo Verde). In this presentation we will highlight the screening efforts we have been doing regarding different applications and activities (e.g. anti-cancer, anti-biofouling, anti-microbial, anti-biofilm, anti-obesogenic and related diseases, cosmetics, food, etc.). LEGE-CC is member of World Federation for Culture Collections (WFCC), European Culture Collections Organisation (ECCO) and it is also part of the Research Infrastructures EMBRC.PT and MIRRI.PT.

**I.1.2. Zebrafish as model for marine biodiscovery and metabolic diseases****Ralph Urbatzka**

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E-mail: [rurbatzka@ciimar.up.pt](mailto:rurbatzka@ciimar.up.pt)**ABSTRACT**

Obesity, diabetes, and steatosis belong to the greatest public health challenges of the 21st century with millions of individuals affected in Europe, but also worldwide. An urgent demand of new compounds is present, and cyanobacteria/microalgae promise to be an excellent source for natural-derived molecules and novel nutraceuticals. Preclinical drug discovery can be improved by the use of phenotypic assays and advanced models, and in particular small whole animal models as zebrafish larvae provide complexity, tissue interaction and safety issues from early on.

In my research group, we have addressed these topics focusing on physiologically relevant models, aquatic resources as cyanobacteria or microalgae, and metabolic diseases. Screening efforts revealed cyanobacterial strains with promising bioactivity in cell-based phenotypic assays, target-based assays, and in physiologically relevant whole small animal screenings using zebrafish larvae for reduction of neutral lipids, insulin mimetics, appetite or intestinal lipid uptake. Toxicity evaluation and metabolite profiling by LC-MS/MS was applied to select promising fractions with high bioactivity, absence of toxicity and potential to produce novel compounds. Novel and known chlorophyll derivatives were discovered with promising lipid reducing activities in zebrafish, and its beneficial effects were confirmed in differentiated murine adipocytes in 3D cell culture. A proteomics methodology was established (bioactive thermal protein profiling, bTPP), and applied to identify the molecular targets.

In the talk, I will give an overview about phenotypic screening and the possible applications of zebrafish in natural product discovery, as well as about research achievements using zebrafish assays for metabolic diseases and cyanobacteria/microalgae.

**Key words:** Zebrafish, biodiscovery, metabolic diseases, natural products.

### I.1.3. Lysosomal reactivation in Atlantic salmon macrophages infected by *Piscirickettsia salmonis*

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**-Introduction:** *Piscirickettsia salmonis*, is an intracellular pathogen responsible of Salmonid Rickettsial Syndrome (SRS), considered as the most important health problem on Chilean aquaculture. *P. salmonis* infects and replicates in salmonid macrophages, inducing an anti inflammatory environment and avoiding its lysosomal degradation. Control and prophylactic strategies against *P. salmonis* have been based on vaccines and antibiotics, which have largely failed. In this work, we propose to evaluate the effect of an immunization strategy based on non-specific antibodies, analyzing the transcriptomic and lysosomal response, cellular viability and bacterial survival in Atlantic salmon macrophages infected with *P. salmonis*.

**-Methodology:** The transcriptomic profile of Atlantic salmon macrophages infected by *P. salmonis* was obtained by a microarray analysis (Salmon Immunity and Quality 15k GPL16555). The lysosomal pH and proteolytic activity were evaluated using LysoSensor Yellow/Blue<sup>TM</sup> and DQ<sup>TM</sup> BSA Green probes, respectively. The effect of non-specific antibodies over cellular viability of infected macrophages was determined by LDH assay and the bacterial load was determined by gentamicin protection assay.

**-Results:** The transcriptomic profile from macrophages infected by *P. salmonis* shows changes in the transcript expression of genes related to the endocytic pathway and lysosomal process. Then, stimulation with antibodies promotes lysosomal activity by lowering lysosomal pH and increasing the proteolytic activity within this organelle. Additionally, incubation with non-specific antibodies elicits a decrease in bacterial-induced cytotoxicity in infected Atlantic salmon macrophages and reduces the bacterial load.

**-Conclusions:** Our results suggest that stimulation of cells infected by *P. salmonis* with non-specific antibodies reverses the modulation of the lysosomal activity induced by bacterial infection, promoting macrophage survival and bacterial elimination. This work represents a new important evidence to understand the bacterial evasion mechanisms established by *P. salmonis* and contribute to the development of new therapeutic strategies against SRS.

**Key Words:** Atlantic salmon, *Piscirickettsia salmonis*, passive immunization, macrophages



### I.2.1. Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital PCR

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**ABSTRACT:** Use of molecular tools in addition to traditional microscopy-based observation has become one of the promising methodologies for plankton monitoring. However, as ribosomal RNA (rRNA) genes are commonly targeted in molecular studies, variability in the rRNA gene copy number within and between species must be considered to provide quantitative information in quantitative PCR (qPCR), digital PCR (dPCR), and metabarcoding analyses. Currently, this information is only available for a limited number of species. The present study utilized a dPCR technology to quantify copy numbers of rRNA genes per single cell in 16 phytoplankton species, the majority of which are toxin-producers, using a newly developed universal primer set accompanied by a labeled probe with a fluorophore and a double-quencher. The copy numbers varied from 44 - 2,031,500 copies cell<sup>-1</sup> in the examined species. The Poisson statistic used for the calculation of copy number per sample accounts for the potential situation when > 1 fragment is contained in the partition, but it is not possible to account for multiple copies per one fragment in one partition. In this study, the influence of the DNA fragment length on the copy number measurements indicated no statistically significant differences between the original fragments (around 10,000 bp) and the larger fragments (9,000, 5,000, 3,000 bp). The length of a single rRNA gene unit varies widely in eukaryotes ranging 7,597-45,306 bp including 577-33,686 bp of the intergenic spacer (IGS) regions. The lengths of 18S + ITS + 28S in the marine dinoflagellate, several *Alexandrium* species are around 5,500 bp. For example, if they have 1,000-3,000 bp of IGS, the lengths of rRNA gene units should be 6,500-9,500 bp. In this study, DNA fragments around 10,000 bp were most abundant in the original DNAs extracted by Chelex, which can potentially explain why the copy numbers were not significantly different from those fragmented in 5,000 and 9,000 bp. Surprisingly, dPCR yielded significantly lower copy numbers in the smaller DNA fragments (1,500 bp) than higher ones, suggesting occurrences of the fragmentation of the DNAs in the middle of the target region. In silico PCR using the newly developed primers allowed the detection of taxa from 8 supergroups, demonstrating universality and broad coverage of the primer set.

**Key words:** absolute quantification; digital PCR, rRNA gene copy number, eukaryotes

### **I.3.1. The machine revolution: development of new antibiotics and diagnostic tests**

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#### **ABSTRACT**

Machines have the potential to outperform humans and revolutionize our world. In this talk, I will describe our efforts to use machines to develop computational approaches for antibiotic discovery, as well as low-cost rapid diagnostics. Computers can already be programmed for superhuman pattern recognition of images and text. In order for machines to discover novel antibiotics, they have to first be trained to sort through the many characteristics of molecules and determine which properties should be retained, suppressed, or enhanced to optimize antimicrobial activity. Said differently, machines need to be able to understand, read, write, and eventually create new molecules. I will discuss how we trained a computer to execute a fitness function following a Darwinian algorithm of evolution to select for molecular structures that interact with bacterial membranes, yielding the first artificial antimicrobials that kill bacteria both in vitro and in relevant animal models. My lab has also developed pattern recognition algorithms to mine the human proteome, identifying throughout the body thousands of antibiotics encoded in proteins with unrelated biological function, and has applied computational tools to successfully reprogram venoms into novel antimicrobials. I will also describe the development of diagnostic biosensors for COVID-19, further substantiating the exciting potential of machine biology. Computer-generated designs and innovations at the intersection between machines and biology may help to replenish our arsenal of effective drugs and generate novel diagnostics, providing much needed solutions to global health problems caused by infectious diseases.

**Key Words:** Machine Biology, synthetic biology, antibiotic discovery, low-cost diagnostics

### **I.4.1. Biotechnological strategies in plants to face the adverse effects of Climate Change**

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#### **-Introduction**

Climate change (CC) causes mainly salinity and drought, known as abiotic stress (AS), generating dramatic damage to agriculture around the world. In Chile alone, a 15-20% reduction in annual precipitation is forecast by 2050, affecting the growth rate and production of crops, including kiwi and tomato, of great relevance to the national economy. Therefore, our objective is to generate new knowledge and biotechnological strategies to improve the tolerance of plants to AS, focusing on tomato and kiwi rootstocks.

#### **-Methodology**

Through literature searches, transcriptomic analysis and preliminary results from the participating laboratories, genes to edit in the tomato and kiwi genomes, and potential bioformulations (bacteria and chemical compounds) to improve the tolerance of these species to drought and salinity were identified, using plants propagated in vitro and under greenhouse conditions.

#### **-Results**

For each negative regulatory gene selected, six orthologous genes were identified in the tomato and kiwi genomes for editing. CRISPR/Cas9 vectors and primers have been designed to monitor and alter the expression of the six genes in kiwi and tomatoes subjected to drought and salinity. Additionally, bioformulations that act as biostimulants and grant tolerance to salinity and drought to tomatoes are being evaluated and generated. For this, *Bacillus amyloliquefaciens* increases the percentage and speed of germination of tomatoes, as well as the vigour of the plants, and rhizobacteria have been identified from the Atacama desert that are tolerant to salinity, with the prospect of being used as biomodulators. Furthermore, the exogenous application of natural antioxidants, such as lipoic acid, positively affects tomato plants subjected to AS.

#### **-Conclusions**

A multifaceted strategy is required to strengthen agriculture in the face of CC challenges. The use of gene editing and bioformulations are part of this strategy. In addition, through various means (eg <https://plantaconciencia.cl/>; Instagram @plantaconciencia), we are improving the understanding and awareness of the public about CC, AS and the biotechnological efforts being used to face them. Funded by ANID PIA ACT192073 and Fondecyt 1181198.

**Key Words:** Abiotic stress, tomato, kiwi, lipoic acid



#### **I.4.2. Recent advances in the study of plants in Chile with applications in neurodegenerative diseases**

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**-Introduction:** This talk will deal with recent studies of endemic plants in Chile with possible applications in neurodegenerative diseases.

**-Methodology:** Enzyme inhibition microplate assays and High Performance Liquid Chromatography (HPLC) coupled to diode array are used, as well as countercurrent chromatography

**-Results:** Isolation results of bioactive compounds and their enzyme inhibitory activity are presented

**-Conclusions:** Several species from Chile produce novel active compounds that are promising for possible treatments of neurodegenerative diseases, such as Alzheimer's and Parkinson's.

**Keywords:** neurodegenerative diseases, HPLC-MS, endemic plants, phenolic HSCCC

### I.4.3. Bioprospecting of plant proteases

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#### ABSTRACT

Enzymes are a biotechnological tool of interest for industry because they are able to achieve a high reaction rate under soft pH, temperature, and pressure conditions, as well as a high specificity of reaction, biodegradability, nontoxic nature, and nonpolluting effluent generation. Proteases are one among the important groups of industrial enzymes, accounting for more than 65% of the total industrial enzyme market and finds in its applications in detergents, leather processes, food processing and pharmaceuticals. Proteases are also envisaged as having extensive applications in the development of eco-friendly technologies and several bioremediation processes. Bioprospection, for new sources of enzymes suitable for application in industrial processes is attracting increasing attention and the raw plant materials to produce enzymes are still interesting.

To advance the understanding and utilization of the regional plant diversity available, it has been proposed to obtain and characterize proteolytic enzymes plant with potential application in various biotechnological processes. In this regard they have been purified and characterized biochemically and structurally proteases obtained from species belonging to different plant families. In order to demonstrate the biotechnological potential of plant proteases, controlled hydrolysis tests of food proteins (soy proteins, caseins and whey proteins) were carried out to generate hydrolysates with improved functional properties and to obtain bioactive peptides. They were also tested in the enzymatic depilation of bovine hides and in terms of their anti-inflammatory activity. The results obtained are very promising for the latex proteases of *Maclura pomifera* (Moraceae), *Vasconcellea quercifolia* (Caricaceae), *Asclepias fruticosa* (Apocynaceae) and *Calotropis procera* (Asclepiadaceae), as well as for the proteolytic preparations obtained from the fruits of *Bromelia hieronymi* and *Pseudananas macrodentes* (Bromeliaceae).

**Key Words:** Enzymes, Plant proteases, Biocatalysis, Enzymatic hydrolysis

**I.4.4. Increased drought tolerance in tomato plants mediated by changes in the metabolism of brassinosteroids****Francisca Parada**

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**- Introduction:** It is expected that the global area suffering water-deficit, and the use of fresh water for agriculture, home use, and industry will increase within the coming decades. Additionally, drought affects crops worldwide, limiting growth and yield, and altering various metabolic and physiological processes. These include decreased relative water content, lower photosynthetic efficiency, oxidative damage, and reduced fruit production. Faced with these productivity problems, one of the strategies that has been used in different species is the exogenous application of plant hormones. Brassinosteroids (BRs) are steroidal plant hormones that regulate various biological functions and have been shown to mitigate adverse effects produced by various abiotic stresses such as cold, salinity, and drought.

**-Methodology:** Tomato transformation will be performed to generate lines with alterations in brassinosteroid metabolism and thus improved drought tolerance. CRISPR/dCas9 technology will be used in which transcriptional activators or suppressors can be fused to the dCas9 protein. This technology changes the expression of target genes without generating alterations in the genome. The vectors will be assembled using the modular system dependent on type II restriction enzymes called "Loop Assembly". Physiological, yield, global gene expression, and BR and metabolite content parameters will be tested in transformed tomato lines.

**-Results:** Bioinformatics analyses in the tomato genome have been performed to find candidate genes from BRs routes for transcriptional activation using CRISPR/dCas9 technology. The tissue-dependent expression profile of genes involved in BRs metabolism will be analyzed during development and drought treatments.

**-Conclusions:** Using the methodology proposed, it is expected that ten improved tomato lines with higher tolerance to water deficit will be obtained. Physiological, transcriptomic, and metabolic analyses of the transformed lines will generate a biological network that integrates all these parameters and explains the phenotype of improved drought tolerance observed in the tomato lines. This work will thus contribute to a more sustainable agriculture. Funded by Fondecyt 3210631.

**Key Words:** drought, tomato, brassinosteroids, CRISPR/dCas9

**I.5.1. A low-cost system for the study of proteins used in salmonid diets, use of proteolysis to determine the quality**

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- **Introduction:** A proteolysis system, a classic biochemistry model, is proposed to enable the feasibility of indirectly assessing the digestibility of protein present in salmonid diets
- **Methodology:** An artificial gastric solution was developed with pepsinogen, the volume of a salmonid stomach was estimated, and 8 g of food was mixed per 50 ml of solution, which was constantly stirred by 3 h and kept at 15° C constant
- **Results:** We note that this artificial gastric solution requires pepsin to degrade samples, which occurs at pH 2.0, in addition, proteolysis depends on time and temperature. Comparing albumin, food and a standard mixture of proteins could observe varying degrees of fragmentation.
- **Conclusions:** The results suggest a low-cost system to evaluate the source of a protein, by correlated the number of fragments generated, indicator of the quality of the protein. In addition, it allows to evaluate mixtures with the disposition of the protein to be digested.

**Key Words:** protein, diet, salmon, gastric



### I.5.2. Effect of reaction conditions on the enzymatic synthesis of isomaltooligosaccharides from forage barley starch (*Hordeum vulgare* L.)

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**-Introduction:** Isomaltooligosaccharides (IMOs) are emerging prebiotics with high commercial demand. They are obtained from liquefied corn starch, but high demand leads to a search for alternative sources of starch. The objective of this study was to determine the reaction conditions that significantly influence the synthesis of IMOs from forage barley starch.

**-Methodology- Experimental design:** The reaction mixture consisted of 15 mL of liquefied forage barley starch (variety UNA La Molina 96) containing 40 ppm of Ca<sup>2+</sup> (as CaCl<sub>2</sub>), adjusted to pH 5.0, and the enzymatic mixture of Promozyme D2, Fungamyl 800L and Transglucosidase L "Amano", which were tested according to the 2<sup>5-1</sup> fractional factorial design matrix. The levels of the independent variables are presented in Table 1.

**Table 1.** Values of the independent variables in the 2<sup>5-1</sup> fractional factorial design.

Independent variables	Symbol	Coded levels	
		-1	+1
Initial starch % (w/v)	X <sub>1</sub>	30	40
Promozyme D2 (IU/g d.m.l.)	X <sub>2</sub>	7	13
Fungamyl 800 L (IU/g d.m.l.)	X <sub>3</sub>	24	94
Transglucosidase L "Amano" (U <sub>T</sub> /g d.m.l.)	X <sub>4</sub>	10	25
Temperature (°C)	X <sub>5</sub>	50	60

d.m.l: dry matter liquefied starch

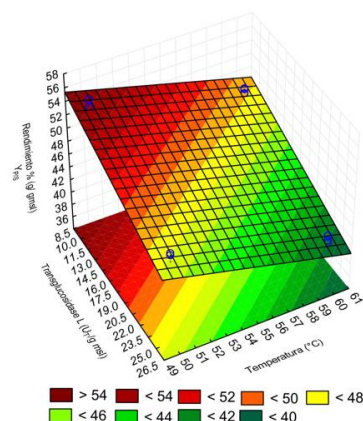
The yield of IMOs was reported as the dependent variable at 26 h. The carbohydrate composition was determined by HPLC. The results of the experimental design were analyzed using Statistica 10.0 software for Windows (StatSoft, Inc. 2011, USA). Differences were considered significant at p-values ≤ 0.05.

**- Results:** The results of the experimental design after 26 h of reaction are shown in Table 2. The yield ranged from 39.8 to 55 % (w/w) under different reaction conditions.

The linear effect of Transglucosidase L "Amano" concentration and temperature on yield is illustrated in Fig. 1. The highest yield values were obtained at the low levels of these variables. This phenomenon is due to the fact that the enzyme Transglucosidase L "Amano" has hydrolysis and synthesis activity and the data show that the rate of hydrolysis of maltose is faster than the rate of

**Table 2.** Results of the 2<sup>5-1</sup> fractional factorial design.

Run	Independent variables					Yield % (w/w)
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	
1	-1	-1	-1	-1	+1	48.8
2	+1	-1	-1	-1	-1	55.0
3	-1	+1	-1	-1	-1	53.5
4	+1	+1	-1	-1	+1	48.0
5	-1	-1	+1	-1	-1	52.9
6	+1	-1	+1	-1	+1	48.7
7	-1	+1	+1	-1	+1	48.1
8	+1	+1	+1	-1	-1	53.9
9	-1	-1	-1	+1	-1	45.8
10	+1	-1	-1	+1	+1	39.8
11	-1	+1	-1	+1	+1	40.6
12	+1	+1	-1	+1	-1	48.2
13	-1	-1	+1	+1	+1	41.0
14	+1	-1	+1	+1	-1	47.9
15	-1	+1	+1	+1	-1	46.5
16	+1	+1	+1	+1	+1	41.8



**Figure 1.** Response surface plot for IMO yield.

formation of IMOs, especially at low concentrations of maltose, which occurs in the course of the reaction. Also, the rate of hydrolysis is more sensitive to the increase in temperature than the rate of synthesis.

**-Conclusions:** The results are promising using forage barley as raw material for the enzymatic synthesis of IMOs and suggest the development of further research focused on optimizing the factors that have the greatest influence on the yield of IMOs.

**Key Words:** isomaltooligosaccharides, forage barley, transglycosylation activity, hydrolysis activity.

**I.6.1. Microorganisms in the generation of acid drainage. Alternatives for their mitigation**

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**ABSTRACT**

The economy of many countries in the world and mainly in Latin America, has metalliferous mining as one of most relevant pillars. The technologies used in large-scale mining make the recovery of metals economically profitable and are usually less polluting than the methods used in small-scale mining. Nevertheless, large-scale tailings are generated mainly because it is necessary to process huge volumes of ore to obtain small quantities of the metals of interest. The impact of these tailings covers different dimensions, but undoubtedly the most serious aspect is the contamination of water resources and soils, which can occur through different routes, among which acid mine drainage (AMD) should be distinguished as the highest risk. AMD is generated by the exposure of sulfide minerals (especially those sulfides such as pyrite) to oxygen and water, which causes their oxidation, usually accompanied by acidification and solubilization of heavy metals in the aqueous medium. These highly acidic, metal-loaded drainages can affect watercourses and ecosystems even at great distances from their source. Although the AMD formation process can develop under abiotic conditions, the presence of iron and sulfur oxidizing microorganisms drastically increases its speed due to the generation of ferric ion, which becomes the main oxidizing agent. There are different physicochemical and biological alternatives to prevent the formation of AMD and/or to buffer its effects on the environment. Limiting the access of oxygen (usually by flooding) is usually an acceptable alternative with some hydrogeological consequences; in addition, the microorganisms mentioned can also act -much more slowly- under anaerobic conditions, so that inhibition of the microorganisms by adding different substances or even antagonistic microorganisms is a more promising alternative. Finally, the treatment of AMD using natural or artificial wetlands would make it possible to reduce the metal load and the acidity of these drains so that they can be discharged with a minimum effect on the environment

**Key Words:** acid drainage; microorganisms; microbial inhibition; bioremediation

**I.6.2. Biology of stress in coastal macrophytes and its uses as biomonitoring tools for environmental diagnosis**

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**-Introduction:** Macrophytes are the basis of trophic networks in coastal areas; thus, their presence is essential for the development and complexity of ecosystems with high ecological and economic value. Depending on the latitude and certain specific conditions of the ecosystems they sustain, these macrophytes can be macroalgae or marine angiosperms. It has been identified that in the face of stress caused by exogenous agents of anthropogenic origin or environmental variations associated with Climate Change, these can show signs of stress at different levels of biological organization, which in addition to assisting in understanding tolerance mechanisms, can serve for the development of biotechnology tools for environmental diagnosis (e.g. functional indicators, biomarkers).

**-Methodology:** Through complementary research on macrophytes combining experiments in a controlled laboratory situation, plus passive and active biomonitoring initiatives in the field, a series of strategies have been developed that, together, allow progress in new protocols for the diagnosis of disturbances associated with pollution and climate change. The latter, from polar to temperate and Mediterranean ecosystems, and focusing on physiological aspects ranging from photosynthetic performance, reactive oxygen metabolism and whole transcriptome analyses (New Sequencing Technologies).

**-Results:** Experiments in a controlled situation on coastal macrophytes have assisted in identifying biological mechanisms and strategies to counteract different stressors, as well as to elucidate their tolerance thresholds and specific responses to vectors of interest. On the other hand, complementary experiments of sampling and transplantation of macrophytes in areas subject to environmental disturbances assist in observing the assertiveness of the biological responses analyzed in the laboratory, and the eventual proposal of these responses for future environmental monitoring programs.

**-Conclusions:** In macrophyte research, ranging from Northern Europe to the Mediterranean Sea, the coasts of the South Pacific Ocean and the Antarctic Peninsula, the ecotoxicological approaches of complementary studies in the laboratory and the field, at different levels of biological organization, provide us with new perspectives and expectations to improve current protocols for environmental monitoring and diagnosis in coastal areas.

**Key Words:** Climate change; desalination, metals; contamination.

**I.6.3. Nature-based solutions for water management****Cristina Sousa Coutinho Calheiros**

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**ABSTRACT**

The increase of population associated with climate change impacts can create innumerable environmental, economic and social problems, around the world. Urban areas have become more impermeable, with an increase fragmentation of habitats, ecosystem degradation, intensification of the heat island effect, water, air and land pollution and consequently leading to deterioration of human wellbeing and disconnection to nature.

The strategy for changing territories dynamics involves the use of Nature-Based Solutions to promote multifunctional areas, operating at different scales and relying on nature to generate environmental (e.g., biodiversity conservation or adaptation to climate change), economic (e.g., job creation and property valuation) and social (e.g., water drainage) advantages. Nature-Based Solutions also play an important role in mitigating the effects of urbanization, aiming to strengthen urban ecosystems in order to be more resilient to the challenges of climate change and contributing to the transition to a low carbon economy. This talk will discuss the integration of Nature Based Solution (NBS) in territories towards managing water resources, offset the rising challenges from water security to population growth and climate change.

**Key words:** nature-based solutions, circular cities, climate change, biotechnology



#### **I.6.4. Enhanced Mutualism Between Soil Phages and Bacteria With Elevated Environmental Stress**

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- **Introduction:** Microbe-virus interactions have broad implications on the composition, function, and evolution of microbiomes. Elucidating effects of environmental stresses on these interactions is critical to identify the ecological function of viral communities and understand microbiome environmental adaptation. Heavy metal-contaminated soils represent a relevant ecosystem to study the interplay between microbes, viruses and environmental stressors.

- **Methodology & Results:** Metagenomic analysis revealed that Cr pollution adversely altered the abundance, diversity and composition of viral and bacterial communities. Host-phage linkage based on CRISPR indicated that, in soils with high Cr contamination, the abundance of phages associated with heavy metal tolerant hosts increased, as did the relative abundance of phages with broad host ranges (identified as host-phage linkages across genera), which would facilitate transfection and broader distribution of heavy metal resistance genes in the bacterial community. Examining variations along the pollutant gradient, phage-bacterium interactions shifted to a more mutualistic relationship in the face of greater environmental stresses. Specifically, the fractions of lysogens in bacterial communities (identified by integrase genes within bacterial genomes and prophage induction assay by mitomycin-C) were positively correlated with Cr contamination levels. Furthermore, viral genomic analysis demonstrated that lysogenic phages under higher Cr-induced stresses carried more auxiliary metabolic genes regulating microbial heavy metal detoxification.

- **Conclusions:** With the intensification of Cr-induced environmental stresses, the composition, replication strategy, and ecological function of the phage community all evolve alongside the bacterial community to adapt to extreme habitats. These results in a transformation of the phage-bacterium interaction from parasitism to mutualism in extreme environments, and underscores the influential role of phages in bacterial adaptation to pollution-related stress and in related biogeochemical processes.

**Key Words:** Soil virome, Microbial mutualism, Auxiliary metabolic genes, Chromium stress

**I.6.5. Microalgae biotechnology applied to the purification of biogas, combustion gases and the use of residual organic effluents**

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**ABSTRACT**

The use of microalgae as part of the treatment of gaseous emissions is a robust and economical option for the control of air pollution. Microalgae grow intensively in tubular photobioreactors, where carbon dioxide (CO<sub>2</sub>) emissions are converted into biomass, an inoculum product for the environment and with possible added value. Experiences at the level of basic research and full-scale applications are presented.

**Key Words:** Biodesulfurization; CO<sub>2</sub> fixation; Nutrients recovery

### **I.7.1. Mitochondrial ncRNA targeting induces cell cycle arrest and tumor growth inhibition of MDA-MB-231 breast cancer cells through reduction of key cell cycle progression factors**

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**- Introduction:** The family of long non-coding mitochondrial RNAs (ncmtRNAs), comprising sense (SncmtRNA) and antisense (ASncmtRNA-1 and ASncmtRNA-2) members, are differentially expressed according to cell proliferative status; SncmtRNA is expressed in all proliferating cells, while ASncmtRNAs are expressed in normal proliferating cells, but is downregulated in tumor cells. ASncmtRNA knockdown with an antisense oligonucleotide (ASO-1537 or Andes-1537) induces massive apoptosis in tumor cell lines, without affecting healthy cells. Apoptotic death is preceded by proliferation blockage, suggesting that these transcripts are involved in cell cycle regulation.

**- Methodology:** MDA-MB-231 breast cancer cells were transfected with Andes-1537 or non-related ASO (ASO-C) or left untreated for 24 h and Western blot was performed with antibodies against key cell cycle progression factors. Levels of microRNAs (miRNAs) were determined by Taqman RT-PCR assay.

**- Results:** Death of breast cancer cells induced by ASncmtRNA knockdown, preceded by proliferative blockage, is mediated by downregulation of the key cell cycle progression factors cyclin B1, cyclin D1, CDK1, CDK4 and survivin, the latter also constituting an essential inhibitor of apoptosis, underlying also the onset of apoptosis. The treatment also induces an increase in the microRNA hsa-miR-4485-3p, whose sequence maps to ASncmtRNA-2 and transfection of MDA-MB-231 cells with a mimic of this miRNA induces cyclin B1 and D1 downregulation. Other miRNAs that are upregulated include nuclear-encoded hsa-miR-5096 and hsa-miR-3609, whose mimics downregulate CDK1.

**- Conclusions:** Our results suggest that ASncmtRNA targeting blocks tumor cell proliferation through reduction of essential cell cycle proteins, mediated by mitochondrial and nuclear miRNAs. This work adds to the elucidation of the molecular mechanisms behind cell cycle arrest preceding tumor cell apoptosis induced by ASncmtRNA knockdown.

**Keywords:** Noncoding RNA; miRNA; Breast cancer; Cell cycle

### 1.7.2. The parity-dependent transcriptome of reproductively aged mouse ovary: prospective markers of ovarian cancer risk.

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#### ABSTRACT

Cancer can be regarded as an age-related disease. In women, reproductive aging is associated to hormone, inflammatory and metabolic changes that predispose to various cancers. In fact, the highest rates of ovarian cancer (OC) incidence and mortality occur at post-menopause. Importantly, OC risk can be modulated by full-term pregnancy history during former fertile life, so that nulliparity increases while parity reduces OC risk. To gain insight on the molecular basis of the long-term effect of parity on OC risk, two cohorts of C57BL/6 mice were maintained under virgin (nulliparous) and multiparous regimens from adult until estropause age. Ovarian RNA was profiled with microarrays resulting in 177 differentially expressed genes (DEGs) between conditions. Functions enriched among genes of higher expression in the aged virgin ovary were glutathione/xenobiotic metabolism, metal ion transport, renin-angiotensin system and cytoskeleton/cell junction. On the other hand, genes of higher expression in the aged multiparous ovary were enriched in oocyte/follicle homeostasis and immune cell chemotaxis. Datamining in GEO, GSEA and TCGA uncovered relevant signaling pathways including PTEN and TGF $\beta$ /BMP as well as gene mutations/amplifications and likely pathogenic/predicted loss of function variants in the human orthologs CLDN11, AARD, AGT and CYP11B1. We conclude that, compared to the multiparous, the virgin mouse ovary approaches a disease-prone senescent state characterized by metal ion flux-related oxidative stress linked to glutathione depletion; ii) activation of the renin-angiotensin system; iii) cytoskeletal and cell junction alterations possibly impacting the ovarian surface; iv) an exhausted ovarian reserve. Results are discussed in their relevance as candidate markers of early OC detection and/or therapy targets in women at risk by nulliparity.

**Key Words:** ovary, cancer, transcriptome, parity, mouse, risk



### I.7.3. Role of proteomics in the identification of metabolic pathways associated with virulence of *Trypanosoma cruzi*

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#### ABSTRACT

Although several molecules have been proposed to be involved in this whole process, the exact molecular basis of *T. cruzi* virulence is not yet understood.

Even that, current studies on *T. cruzi* virulence have focused on comparing the *T. cruzi* virulence between virulent and avirulent strains and isolates, however, no conclusive evidences have been reached respect to the molecular bases of virulence. The later can be attributed to the heterogeneity of the samples used, and that those parasites differ greater on its genetic background of the DTU isolates, and have been isolated from different hosts, from different geographical areas. Then, the search for a good model to study *T. cruzi* virulence is strongly required to determine the role of the different virulence factors in a global way, considering also that virulence could not only be associated with the higher or lower expression of a molecule or set of molecules but also with the expression of the metabolic pathways associated with their expression including also those related to energy production. Then, the study the virulence among *T. cruzi* cell lines originated from the same clone with opposed virulence phenotype could provide a better model to understand the molecular basis of these differences. Then, two aliquots from *Trypanosoma cruzi* clone H510 C8C3 were cultivated in a different way for thirty years, generating a virulent cell line (C8C3hvir) and other of low virulence (C8C3lvir). In order to explore differences in both cell lines a LC-MS/MS, was developed and a gen ontology analysis was carried out. This approach showed a total of 1547 proteins, of which 387 showed differential expression in C8C3hvir with respect to C8C3lvir. Among these, 174 were positively regulated while 216 were negatively regulated. Principal component analysis of the positively regulated proteins showed that proteins for dissemination or transmission of organisms from other organisms (within biological processes), ribosomal proteins (within cellular components) and succinyltransferase activity (within molecular functions) were the most overexpressed. It is concluded that the C8C3hvir cell line not only differentially overexpresses some virulence factors with respect to the C8C3lvir line, but also some proteins of biological processes, cellular components and molecular functions, all of which, beyond the expression of a single virulence factor, are part of a genetic program designed to invade, survive in the host and eventually cause damage

**Key Words:** Proteomics, virulence, *Trypanosoma cruzi*

**I.7.4. mRNA-Based Vaccines: Considerations and Solutions for Successful Manufacturing****Lucas Gentilini**

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During 2020, the health emergency caused by the SARS-CoV2 virus around the world generate the requirement to develop new generation vaccines to be able to supply the demand in a timely manner. Among the technologies that were promoted are mRNA-based vaccines, an immunization strategy against viral infections not approved until then for human therapeutic use. In order to understand this technology in detail, the different technical aspects of the mRNA-based vaccine manufacturing will be shared during this conference, with particular emphasis on the technological challenges that this vaccination strategy presents and how they can be faced and solved in order to guarantee a safe and robust production.

**Key Words:** mRNA, Vaccine, bioprocess, COVID

### **I.7.5. Upstream and downstream processing of cell culture-derived virus particles**

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#### **ABSTRACT**

The production of viral particles derived from cell cultures intended for obtaining biologicals such as vaccines, has generated great attention due to the global pandemic that afflicts the world since 2019. Biological production involves many steps that are known as Upstream (USP) and Downstream (DSP) process, which are technical terms that grouped different stages. The USP is related to the management of the biological component or producer of viral particles, which include since the management of cell and viral banks, whose cultures must be expanded in closed controlled systems known as bioreactors until the viral-harvest step. In this stage, it is sought to obtain a good productive efficiency which can be achieved after optimizing the Bioreactor conditions (physical and biological parameters) or adaptations of the system (batch or continuous) to produce high cell - densities with high viral titers in a short term. On the other hand, the DSP consists of steps that focus on the separation, purification, inactivation, and filtration of the biological product before the packing stage. In this process, the optimization at the purification level is desired, which seeks viral-recovery values around  $10^6$  to  $10^8$  viral particles, with fewer contaminants (Host DNA (0.01%) and low quantity of proteins (25 µg per dose)) using different combinations of chromatographic columns. Many USP and DSP optimization strategies are described throughout the literature; however, the best option is related to the balance of benefits and costs. Despite the complexity of these processes, USP and DSP are only part of 30% of a regular vaccine production process and the main percentage is related to the Quality Control Steps (QC), which are important to validate the general production of a biological and compliant the GMP (Good Manufacturing Practice) regulations. This overview describes the main optimization strategies for processes related to the biological production of viral particles, employing as a reference the Influenza and the VERO cell line (African green monkey kidney cells) as models.

**Key Words:** Bioprocessing, upstream, downstream, vaccine

### I.8.1. Behind the secrets hidden at the end of the thread

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**Introduction:** Eukaryotic chromosome ends or telomeres are made up of short repeated sequences, rich in TG/CA; toward their centromere-proximal end, there are telomere-associated sequences (TAS), which are longer than telomere motifs, moderately repeated, and species-specific. Telomeric DNA is mostly replicated by reverse transcription, using an enzyme composed by RNA/protein called telomerase. The understanding of the replication mechanisms of telomeres and their associated metabolism, steered scientists to discover that telomerase activity is constitutive in unicellular eukaryotes, and that has a role in the control of cell replicative potential, in metazoan life-extension and cancer development. Here we present the progress of *Ustilago maydis* as an alternative fungal model that experiences cell differentiation. It is suitable to manipulate and has long been developed by scientists around the world for other approaches of high-level research; the beginning and the prominent findings on the chromosome-ends metabolism in this fungus are also presented.

**Methods:** The strains of *U. maydis* are 521 and 518, donated by Dr. W.K. Holloman, and its telomerase-negative mutants trt1-1, trt1-2, ter114-2, and ter114-24. The methods used include extraction of ac. nucleic, PCR, cloning, PFGE, and conventional agarose electrophoresis, Southern blot, one-step gene disruption, in-planta cross, transcriptome analysis.

**Results:** The two main subunits of telomerase from *U. maydis* were identified, partially characterized, and disrupted. The resulting mutants trt1-1 trt1-2, ter114-2, and ter114-24 showed similar phenotypes to those of analogous mutants est2 and tlc1 of *Saccharomyces cerevisiae*. In *U. maydis* telomerase activity was essential for genomic stability and to complete the fungus's cycle of life. Transcriptomic analysis showed the expression pattern of mutants described and others that are specific to this fungus.

**Conclusions:** We have contributed to the development of *U. maydis* as a model organism for telomeric DNA replication. The central components of telomerase and the mechanisms of telomere replication have similarities with those of other fungi and also with those of humans, the global transcriptional expression has been outlined telomerase absence. *U. maydis* promises to be a useful system to carry out complementary studies of the primary metabolism of telomeric DNA.

**Keywords:** *Ustilago maydis*, telomerase, model organism, telomere



**I.8.2. Detection of *Toxoplasma gondii* by qPCR in clot and cerebrospinal fluid samples from patients with HIV/AIDS**

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**Introduction:** Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*, an obligate intracellular parasite that infects warm-blooded animals including humans as intermediate hosts and felids as definitive hosts. Humans are infected by consumption of food and water contaminated with oocysts of the parasite, or vertically from mother to fetus (congenital toxoplasmosis). The distribution and prevalence of toxoplasmosis depends on the environment and habits of the population. Toxoplasmosis is problematic in populations at risk such as pregnant women and immunosuppressed persons (HIV/AIDS patients). In immunosuppressed persons the most severe and common manifestation is toxoplasmic encephalitis, which occurs through reactivation of chronic toxoplasmosis. Traditionally the diagnosis is made by serological methods, and direct observation of the parasite in fluids or tissues (with low sensitivity and specificity). Alternatively, a clinical diagnosis can be corroborated with radiological images and observation of response to treatment. Recently, molecular tools such as quantitative polymerase chain reaction (qPCR) performed on different biological samples have improved the sensitivity and specificity to detect the pathogen, particularly when multicopy genes such as REP529 are targeted. **Method:** 116 patients were enrolled between years 2016 – 2020 from the Regional Hospital of Loreto. Inclusion criteria consider patients older than 18 years with HIV and neurological syndromes. Blood clot and cerebrospinal fluid (CSF) samples were collected. DNA was extracted using a commercial kit and the quantity and quality of DNA was measured (NanoDrop2000). qPCR was performed using primers and probe targeting the 200-300-fold conserved and repetitive region in the parasite genome (REP529). **Results:** 8.62 % (10/116) positive patients were detected using the clot sample, while 27.6% (32/116) were detected using CSF. The average Cq obtained from clot samples was 32 (29-36.5) detecting between 1 to 100 eq-pair/ml. The average Cq obtained from CSF samples was 28 (24.4-36.8) detecting between 1 to 1000 eq-pair/ml. **Conclusions:** The sample with the best sensitivity to detect *Toxoplasma gondii* by qPCR in immunosuppressed patients is CSF.

**Key Words :** Toxoplasmosis encephalic, Molecular techniques, zoonosis.

### **I.8.3. HuR Reduces Radiation-Induced DNA Damage by Enhancing Expression of ARID1A**

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**-Introduction:** HuR is an RNA-binding protein that mainly binds the 3' untranslated region (UTR) of its target mRNAs to regulate their stability and/or translation. Through these actions, HuR regulates several cellular processes, including cell proliferation, the response to DNA damage, and cell survival. Elevated expression of HuR is found in tissues of virtually all forms of cancer, regulating the stability of many mRNAs that encode cancer-related proteins, thereby promoting survival, proliferation, angiogenesis, and invasion. ARID1A, a subunit of the chromatin remodeling complex SWI/SNF, regulates cell cycle progression, interacts with the tumor suppressor TP53, and prevents genomic instability. Because it has been shown to promote non-homologous end joining (NHEJ) DNA repair, ARID1A represents a promising therapeutic target to sensitize cancer cells to chemotherapy and radiation. We hypothesize that HuR, by binding and stabilizing ARID1A transcriptional product, promotes the expression of ARID1A during radiation resistance in triple negative breast cancer cells

**-Methodology:** We used several molecular biology approaches, such as RNP-IP, half-life assays, and genetic and pharmacological inhibition of HuR, to demonstrate that ARID1A is a bona fide target of HuR. We also used clonogenic assays to show that ARID1A acts as a radio protector in TNBC cells. We used western blot analyses and comet assays to evaluate the role of ARID1A in response to radiation-induced DNA damage.

**-Results:** Our results indicate that HuR binds ARID1A mRNA, thereby increasing its stability and thus promoting its expression in breast cancer cells. We further find that ARID1A expression suppresses the accumulation of DNA double-strand breaks (DSBs) caused by radiation and can rescue the loss of radioresistance triggered by HuR inhibition,

#### **-Conclusions:**

1. We report that ARID1A is regulated by HuR.
2. Our results suggest that ARID1A plays an important role in HuR-driven resistance to radiation.
3. Our work shows that HuR and ARID1A form an important regulatory axis in radiation resistance that can be targeted to improve radiotherapy in breast cancer patients.

**Key Words:** Radiation, TNBC, ARID1A, HuR

**I.8.4. The nuclear transcription factor, NR4A3, is required for Plasmodium exoerythrocytic infection**

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**ABSTRACT**

Human nuclear hormone receptors are an important class of drug-able therapeutic targets that are involved in both intra and intercellular signaling. These receptors bear a DNA binding domain that activates transcription, and a ligand binding domain that interacts with small molecules, such as hormones. Recently, a dual RNAseq study of malaria parasite-infected hepatoma cells showed that the human orphan nuclear receptor, NR4A3, is transcriptionally upregulated during parasite exoerythrocytic stage infection. Here we show, using quantitative RT-PCR, that NR4A3 transcripts increase after malaria parasite infection in different hepatoma cell lines, as well primary hepatocytes. We further show that this transcriptional upregulation is accompanied by an increase in protein expression, as detected by Western blot in cells infected with *Plasmodium berghei*, and by immunofluorescence microscopy in cells infected with *P. vivax* and *P. berghei*. We used CRISPR-Cas9 technology to disrupt the NR4A3 gene in the hepatocytes (HC04 cells) and these edited cells were no longer able to support parasite development: HC04-NR4A3 wildtype cells are readily infected with *P. berghei* and *P. vivax* exoerythrocytic forms (EEFs), as we found an average 9.84 *P. vivax* EEFs per well (both hypnozoites and schizonts are observed), but infections are dramatically reduced or eliminated compared to an average of 0.04 *P. vivax* EEFs per well in HC04-NR4A3 KO cell lines. Our data present a novel, host-directed strategy for preventing malaria.

**Key Words:** NR4A3, Plasmodium, malaria, CRISPR/Cas9

**I.8.5. Development of anti-human IgM small fragment antibodies (nanobodies) as universal reagents for immunodiagnostic of infectious and noninfectious diseases**

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**ABSTRACT**

Nanobodies are the smallest antibody fragments which bind to antigens with high affinity and specificity. Due to their outstanding physicochemical stability, simplicity and cost-effective production, nanobodies have become powerful agents in therapeutic and diagnostic applications. In this work, the advantages of nanobodies were exploited to develop generic and standardized anti-human IgM reagents for serology immunoassay and analysis of IgM+ B-cell in human blood. Selection of anti-IgM nanobodies was carried out by evaluating their production yields, stability, binding kinetics and cross-reactivity with other Ig isotypes. High affinity nanobodies were selected with dissociation constants (KDs) in the nM range and high sensitivities for detection of total IgM by ELISA. The nanobodies also proved to be useful for capturing IgM in the serodiagnosis of acute infections through the development of an immunoassay for detection of dengue virus specific IgMs in serum from patients. Finally, due to the lack of an Fc region, the selected nanobodies do not require Fc receptor blocking steps, facilitating the immunophenotyping of IgM+ cells by flow cytometry, an important means of diagnosis of immunodeficiencies and B-cell lymphoproliferative disorders. This work describes versatile anti-IgM nanobodies that, due to their recombinant nature and ease of reproduction at low cost, may represent an advantageous alternative to conventional anti-IgM antibodies in research and diagnosis.

**Key Words:** Immunoglobulin IgM, Nanobody, Immunoassay/ELISA, Serological diagnosis



### **I.9.1. The Human Microbiome: from Evolution to Biotechnological Applications**

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#### **ABSTRACT**

The human microbiome (i.e., the collection of organisms and genes living on and inside us) plays a relevant role in health dynamics. In the gastrointestinal tract, the microbiome has a strong relationship with health, disease, pathogens, and diet, among other factors. Microbial communities living in the gut have ecological properties common to different environments, such as competition or cross-feeding, leading to various dynamics involving horizontal gene transfer, purifying selection, and genetic diversification. The comprehension of the evolutionary dynamics of the gut microbiome members can help us gain insights into the origin, adaptation, and success mechanisms of several species and how those changes may impact the host. In these cases, the use of comparative genomics and phylogenetics, in addition to functional annotation and comprehensive analysis, can help us to present functional models, allowing us to obtain a better understanding of the evolutionary dynamics of different species of the human microbiome.

This presentation shows studies about the genomic evolution of two relevant taxonomic genera of microbes associated with the human gut. The first study focuses on the taxonomic and genetic diversity of *Blautia*, an important and abundant group of the human gut microbiome. The other example is *Akkermansia*, an emergent probiotic with a promising role in metabolic control. In this work, four relevant aspects are mainly presented about the evolution of microbial genomes in the human microbiome: 1) the diversity of species is much higher than the proposed from classical taxonomic methods; 2) the genetic content in some groups represent a complex diversification pattern in the microbiome, reflected by the existence of "open pangenomes"; 3) there are a considerable number of genes in these genomes with a pattern of horizontal transfer, mostly from other members of the microbiota; and 4) very massive gene gain/loss events during the evolutionary history of the genus may lead to new abilities and specific specializations. The use of this information may lead to the formulation of potential biotechnological applications, such the search for new probiotics or the detection of postbiotic agents.

**Acknowledgements:** ANID Initiation Grant #11200209 (Chile)

**Key Words:** Microbiome, Phylogenomics, *Blautia*, *Akkermansia*

**I.9.2. Search for new antimicrobial substances or mechanisms against multi-resistant pathogens: the case of *Pseudomonas aeruginosa* and lactic acid bacteria**

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**- Introduction:** *Pseudomonas aeruginosa* is an opportunistic pathogen that is capable of generating a wide range of infections in diverse hosts. On the other hand, *Lactobacillus spp.* groups lactic acid bacteria where there are species with evident probiotic properties and antimicrobial activity against human pathogens. In the present work, the antimicrobial potential against *P. aeruginosa* of *L. kunkeei*, a fructophilic bacterium present in the gut of honey bees, was studied.

**- Methodology**

- 1) Quantification of *P. aeruginosa* biofilms grown individually or in the presence of *Lactobacillus spp* cultures were performed by Crystal Violet staining.
- 2) Analysis of the protective potential of *Lactobacillus spp* cultures against *P. aeruginosa* infection in a *G. mellonella* model.
- 3) Phenotypic study of the presence of an antimicrobial potential against *P. aeruginosa* by plaque inhibition assays and growth curves.

**- Results:** The results showed that, compared to other *Lactobacillus* species analyzed, *L. kunkeei* had a greater capacity to inhibit the formation of *P. aeruginosa* biofilms in vitro and in vivo and that it was capable of generating antimicrobial substances when co-cultivated together with the pathogen.

**- Conclusions:** *L. kunkeei* isolated from honey bee intestine has the ability to inhibit the growth and biofilm formation of human pathogens, in this case, *P. aeruginosa*.

**Key Words:** Antimicrobials; *Lactobacillus kunkeei*; biofilms; *Pseudomonas aeruginosa*

### I.9.3. Exploiting cyanobacterial metabolism for the discovery of new natural products

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**- Introduction:** Cyanobacteria are a diverse group of photosynthetic prokaryotes and efficient producers of natural products, both characteristics very appealing for biotechnological applications. However, the full potential of cyanobacteria is still underexplored considering the large number of secondary metabolite biosynthetic gene clusters whose products are yet to be discovered. Among these putative natural products, compounds incorporating fatty acid moieties are particularly common in cyanobacterial genomes comparing to other microorganisms.

**- Methodology:** In this study, we exploit the apparent lack of a functional beta-oxidation pathway in cyanobacteria, combining supplementation of cyanobacterial cultures with deuterated fatty acids with mass spectrometry, which allowed an efficient stable-isotope labeling of the fatty acid-derived lipidome.

**- Results:** We showed that this strategy can be used to easily detect natural product signatures in individual strains. The utility of this strategy is demonstrated in two cultured cyanobacteria by uncovering analogues of the multidrug-resistance reverting hapalosin, and novel, cytotoxic, lactylate-nocuoilin A hybrids – the nocuolactylates.

**- Conclusions:** This work represents a new strategy for the discovery of natural products in cyanobacteria. Although limited to a subset of compounds, this strategy enables efficient and fast discovery of fatty acid derived metabolites, which are abundant in cyanobacteria. It enables the targeted deorphanization of FA-incorporating BGCs of interest but also untargeted discovery to swiftly screen multiple strains for isolation targets. By applying this method to two cultured strains, we detected and elucidated the structure of five new hapalosin analogues as well as a family of new cytotoxic lipids, the nocuolactylates.

**Key words:** Cyanobacteria; Natural Products; Fatty acids; Beta-oxidation

### **I.9.4. Increasing biomolecules in microalgae while preserving growth rates. Is it possible?**

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#### **ABSTRACT**

Microalgae are organisms that have important ecological functions, the most impressive is their role as primary producers and base of aquatic food webs, thus supporting a whole range of organisms. This is possible because they have high productivity of biomass and, consequently, of organic molecules that are part of their structure. The commercial interest on these molecules have arisen in reason to potential applications in the pharmaceutical, nutraceutical, cosmetic and other industries, allied to the fact that microalgae have microbial attributes as fast growth rates, possibility of being kept in closed photobioreactors, and morphological and physiological plasticity. This last characteristic is of particular interest because it may result in an even higher biomolecules synthesis that can be induced under some specific growth conditions. Literature has shown that when a microorganism senses a stressing situation, which may cost species survival, the physiological plasticity comes into action and some biomolecules are synthesized at higher rates than usual and accumulate inside the cell. However, this usually occurs in the expense of cell mitosis and generation of new organisms, resulting in lower growth rates as well as lower biomass. In a biotechnological perspective, one apparent possibility to overcome this problem is to culture the microorganism in two sets of batch photobioreactors, the first in ideal growth conditions and the second under the specific stressing situation. However, this requires the transference of biomass (or alternatively of the medium) to the new, stressing, condition. There are some exceptions that makes the process not complicated, for example if light is the desired stressing, but if it is a chemical agent that must be removed, as a macronutrient, then it gets more difficult. We know that macronutrients are needed for structural biomolecules and without them growth is hampered, but micronutrients are used for protein activation, electron carriers in photosynthesis and other metabolic functions. Because they affect some specific metabolic routes, are required in diminute amounts, and can be added to the cultures, its use as stressing factor can be advantageous. Here we will talk about a process that, if precisely selecting a micronutrient concentration and understanding microalgae physiological response, we are able to increase the synthesis and accumulation of biomolecules without affecting its growth rate in great extent and gaining in productivity. Case studies with copper as micronutrient will be presented and discussed in the light of microalgae physiology.

**Key words:** algal physiology, biomolecules, biomanipulation



**I.10.1. Mining Biotechnology in the development of antiviral solutions during the Covid19 pandemic****Mario Esparza Mantilla**

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Mining biotechnology applies microorganisms or their products to extract valuable elements from a solid organic, inorganic (minerals) or mixed matrix to generate goods or services with added sustainable development. In recent decades, mining processes have incorporated clean metal extraction technologies, recovery of RAEs, where copper, gold and silver metals are recovered cleanly with microorganisms, enhancing the circular economy. In the Covid19 Pandemic, industrial copper mining operations have increased their production capacity and demand, with the value of copper being the highest since the previous H1N1 pandemic. Copper has been confirmed as the antiviral metal against SARS-Cov-2, as it is capable of inactivating it in a few minutes, in viral culture models with human cells. This has allowed copper to be applied in Biosafety devices to minimize the transmission of SARS-Cov2. Consequently, the mining biotechnology model for the extraction of metals needs to be strengthened with the insertion of more advanced human capital, an increase in technological suppliers of leachate biomass, a flexible legal framework for biosafety bonds based on clean copper, and the strengthening of academia with the industry. The challenges for biotechnology in times of pandemic are to integrate biotechnology in health and mining biotechnology to accelerate containment processes against human (viral) pathogens and mitigate their transmission, in this work the different contributions of mining biotechnology, models of antiviral copper and the circular biosafety model against the covid19 pandemic.

**Keywords:** covid19, copper, bacteria, virus, biotechnology

### I.11.1. Obtaining and Characterizing Cellulose Nanofibers from waste of the orange juice industry using Ionic Liquids

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**- Introduction:** Different sources of lignocellulosic biomass are produced worldwide, reaching an estimated 1.3 billion tons/year, while in Brazil this value reaches approximately 350 million tons/year. In this context, it is possible to highlight orange (*Citrus sinensis*), considering that the country is responsible for a production of approximately 19 million tons, which corresponds to, approximately, 39% of all world crop and puts Brazil at the highest producer level. Since the chemical composition of these residues consists mainly of cellulose, hemicellulose, and lignin, these components can be fractionated and extracted for the synthesis of value-added products such as ethanol, enzymes or even cellulose nanofibers. The current paper presents a proposal to evaluate an alternative method that can substitute the acid hydrolysis as the main means to obtain cellulose nanofibers, for a method that presents better or equally efficient, economical, and less polluting. In order to obtain this, the lignocellulosic residue of the orange juice industry is being used, which has significant productivity in several countries, mainly Brazil, which stands out as the main producer.

**-Methodology:** 1.- Chemical Treatment (Ionic Liquids) - The residue of the biomasses in the 32 mesh granulometry was treated with the three different ionic liquids. For this purpose, 1:10 m/v (residue /L -1) was subjected to heating at temperatures of 70 °C and 90 °C for 2 h with an agitation of 1000 xg. 2.- Purification of nanocelulose - The suspensions resulting were subjected to filtration and centrifugation at 10,000 xg for 10 min, giving the following fractions: Solid, Supernatant and Precipitate. 3.- Biomass chemical and physico-chemical characterization - Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis, X-ray Diffractometry and Scanning Electron Microscopy (SEM).

**- Results:** Data showed typical structural characteristics of cellulose and were consistent with the nanocellulose extracted from other sources in FTIR analyses. This fact can be evidenced through the events associated with endothermic reactions in the cellulose mass loss region, suggesting that in the precipitated fraction there is a higher concentration of cellulose nanofibers in TA analyses. Although peak  $2\theta = 35^\circ$  from DRX was observed more intensively in the cellulose control, however, it is possible to identify the same peaks at low intensity, which may mean that the treatment with the ionic liquid provided some interference/removal in the amorphous domains of the material, further exposing the crystalline domains. In the SEM micrographs it is possible to clearly observe the structure of the cellulose nanofibers formed from the proposed treatment, which are arranged forming an irregular network of fibers with micrometers of length and diameter that can vary between 70 and 80 nm approximately.

**- Conclusions:** The treatment with the ionic liquids was effective in the process of decontamination of the lignocellulosic matter and the consequent production of the cellulose nanofibers.

**Key Words:** Lignocellulosic residue; Ionic Liquids; Cellulose Nanofiber.

### **I.12.1. Preventive Diagnosis of Diabetic Foot using Smartphone (StandUp H2020 Project)**

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- **Introduction:** Temperature of the plantar foot surface is an important feature in type II diabetes as abnormal temperature variations can be an early sign of foot diseases. In this paper, automatic way to analyze these temperature variations is presented by using an infrared camera. A robust acquisition protocol is proposed and an image processing software is developed.

- **Methodology:** Three types of analysis are performed. First, the mean plantar foot temperature of both feet results from a segmentation procedure based on the Chan and Vese active contour method. Second, the point-to-point absolute mean difference between the 2 feet is assessed by using a rigid registration method. Third, significant hyperthermia regions such that the point-to-point absolute difference is greater than  $2.2^{\circ}\text{C}$  are highlighted. A second study is composed of two main contributions. The first one concerns the segmentation of plantar foot thermal images. It consists of using the deep learning method U-Net to segment the thermal images. The second part is devoted to a transversal clinical study conducted within the Hospital National Dos de Mayo in Lima, Perú.

- **Results:** 82 type II diabetic subjects in a preulcerative state were recruited in the Dos de Mayo hospital (HNDM) in Lima, Perú. These persons were classified in two risk groups of developing an ulcer based on a medical exam: a medium risk group, and a high risk group. Results show that the mean temperature of the plantar foot surface is higher of  $1^{\circ}\text{C}$  in the high risk group compared to the medium risk group. The mean point-to-point absolute difference shows identical values in the 2 groups. Finally, 9 subjects out of the 82 ones show significant hyperthermia of one foot compared to the other (6 in the medium risk group and 3 in the high risk group).

- **Results of a second study:** 122 type II diabetic patients without ulcer were recruited. These individuals were classified into three risk groups of developing a foot ulcer. This classification is based on a medical examination: a low-risk group (R0), a medium-risk group (R1) and finally a high-risk group (R2). The study reveals that the average temperature of the plantar foot is  $1^{\circ}\text{C}$  higher in R1 than in R0 ( $p < 0.1$ ). The R1 group patients are characterized by a rapid recovery of their initial temperature after the cold stress test, compared to R0 and R2 ( $p < 0.01$ ).

- **Conclusions:** These results demonstrate that thermal camera temperature assessment could help in the preventive diagnosis of diabetic foot.

**Key Words:** Diabetic foot, thermal imaging, segmentation, mobile health.

### **I.12.2. Medicine Based Engineering and Informatics to Foster Patient Physician Relationship**

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#### **ABSTRACT**

Biomedical Engineering and Medical Informatics can indirectly better patient physician relationship by new instruments and software applications designed by interdisciplinary teams. The necessity of pregnancy, labor and new born data to lower maternal and neonatal mortality in the 1980s gave rise to the Perinatal Information System, SIP, and Personalized Perinatal System, SEPEPE, in the 2020's. A similar personalized follow up cardiac failure system, SIMIC, confirms the innovative concept of Prescription App. The need to avoid lower limb lesions during rehabilitation prompted DINABANG as a force and velocity portable instrument to be used in the sports field. Naive translation of information systems rationale to clinical use as Electronic Clinical Records still finds resistance to adoption, due to lack of interdisciplinary design. A disruptive innovation is needed to help physicians to take notes with no templates but rather automatic reminders of similar cases, reducing time-to-diagnostic and minimizing errors/oversights. Examples of technology developed from clinical perspective are ABDOPRE, an automatic control vacuum bell over the abdomen to treat intraabdominal hypertension with vesical catheter as control variable, PRAXIS to capture the single physician's case mix to easily solve future patients with the help of reasoning sequences and NEFROVOL as a non invasive measure of polycystic kidney volume. Technology transfer is the epilogue of research, described with the examples of pulmonary mechanics measurement instrument MECVENT, hyperbilirrubinaemia reduction lamp BiliLED and portable lower limb kinetics meter DINABANG. The lessons learned for a successful commercialization are (i) patent owners should work full time, (ii) incubation and support is secured and (iii) specification/development of software or device are done by an interdisciplinary team. Biomedical Engineering and Medical Informatics converge on a broad interdisciplinary area that could be identified as Medical Engineering.

**Key Words:** Biomedical Engineering, Medical Informatics, Biomedical devices, Software enhancements of Medicine



### **I.13.1. Development of llama nanobodies against the spike protein of the SARS-CoV-2 virus and neutralization of the virus in cell culture**

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**Introduction:** The infection by the new coronavirus SARS-CoV-2 causes the COVID-19 disease which originated in China in 2019. Currently it spreads worldwide in different countries and has motivated the declaration of a global health emergency.

Having an effective treatment against COVID-19 will allow the affected people to be cured or improve the chances and recovery times of patients; reducing the costs of care and reducing the chances of infection.

**Methodology:** An immune library of nanoantibodies was constructed by immunization of a llama (*Lama glama*) with complete and inactivated virions of the SARS-CoV-2 virus, from which a library of nanobody VHH genes was constructed and specific nanoantibodies against spike protein selected by phage display. Selection of specific clones against the spike protein resulted in 24 clones. The ability of nanobodies to neutralize virus infection in VERO cell culture was evaluated.

**Results:** The humoral immune response of llama showed an increase in specific antibodies against the complete virions of SARS-CoV-2. The selected nanobody clones were recombinantly expressed in *E. coli* and affinity purified. The neutralization test showed that both the immune serum after immunization, as well as some of the nanoantibodies against spike protein can neutralize the virus infection in cell culture.

**Conclusions:** Our results demonstrate the efficacy and usefulness of nanobodies to neutralize the SARS-CoV-2 virus in cell culture, opening the possibility of expanding the studies in hamster animal models with a view to evaluating their antiviral capacity against the SARS-CoV-2 virus.

**Key Words:** COVID-19, SARS-COV-2, nanobody, spike protein

### II.1.1. Molecular Imprinting of Coronavirus Attachment Factors to Enhance Water Quality Monitoring and Treatment

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**- Introduction:** The COVID-19 pandemic (caused by SARS-CoV-2) might be a dress-rehearsal for even more environmentally transmissible (e.g., waterborne) diseases that are very difficult to control. While the detection of SARS-CoV-2 RNA in wastewater influents has been a powerful tool to inform decision making through wastewater-based epidemiology efforts, detection of viral RNA does not give information on the potential virulence of coronaviruses in these wastewater streams. Additionally, coronavirus infectivity assays cannot detect specific strains of coronaviruses, as can detection of RNA through quantitative reverse transcription PCR (RT-qPCR), which would complicate positive findings. Thus, there is an urgent need for virus detection methods that are strain specific and provide information on the infectivity. This will inform whether more precise viral disinfection approaches are needed to enhance water treatment to protect public health.

**- Methodology:** We employ molecular imprinting, which uses an enzyme-substrate like “lock and key” principle, to create molecularly imprinted polymers (MIPs) that can selectively adsorb infectious coronaviruses. By varying the molecule that we imprint into the polymer we can enhance the specificity of the material for a certain strain of coronavirus. The particles that we imprint onto are highly versatile in application and can be dosed into water treatment processes as adsorbents that easily settle from solution or can be coated onto surfaces for applications such as sensing or filtering.

**- Results:** We suspended the infective coronavirus of interest in aqueous solution with the MIPs (or non-imprinted polymers) in a continuously mixed batch reactor. These adsorption tests show that the material, whether imprinted or not, can achieve nearly instant adsorption (~1 minute) resulting in greater than 1-log removal of coronavirus particles. Additionally, by comparing the effect of imprinting different molecules, we successfully developed a MIP that can achieve 3-log removal of coronavirus within 30 minutes.

**- Conclusions:** In this work we show that MIPs can be used to selectively remove coronaviruses from aqueous solution. By comparing various templates for molecular imprinting, we provide a proof of concept for a highly versatile imprinting strategy that can be used to rapidly develop materials for emerging biological pollutants of concern.

### **II.2.1. Chitosan-based hydrogels for application in bone tissue engineering**

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**ABSTRACT:** Repair of bone defects by bone tissue engineering (BTE) is considered as an alternative to conventional grafts. Hydrogels, an insoluble polymer 3D networks, are very interesting in biomedical applications like tissue engineering as well as drug delivery due to their unique characteristics: biodegradability, ability to mimic the extracellular matrix and capacity for controlled release of bioactive molecules. The aim of this research was to develop hydrogels based on polyelectrolyte complexes by mixing chitosan (CHT, cationic) and polymer of cyclodextrin (PCD, anionic) in order to elaborate two types of BTE scaffolds: an injectable hydrogel and a sponge. The formulation of CHT/PCD hydrogels was performed by tuning the composition ratios of two PCD components: soluble-form PCD (PCDs) and insoluble-form PCD (PCDi). Briefly, CHT/PCD hydrogels were prepared using a system of two interconnected syringes, one with co-milled CHT:PCD powders and the second one with ultrapure water. After mixing, a specific quantity of lactic acid was added into the mixture in order to obtain the hydrogel. For the injectable CHT/PCDi/PCDs hydrogels, optimization of composition ratio was based on evaluating their rheological properties, injectability and cytotoxicity. The beneficial effect of combining both PCDi and PCDs in the hydrogel was clearly observed on the properties of hydrogel. Namely, the CHT/PCD hydrogel, composed of equal quantity of PCDi and PCDs, demonstrated the best compromise between structural stability, shear-thinning and self-healing properties, and injectability. An excellent cytocompatibility with pre-osteoblast cells (MC3T3-E1) was also confirmed for the hydrogel. CHT/PCD sponges were obtained by freeze-drying CHT/PCD hydrogels, followed by a thermal treatment and characterized by their physicochemical, mechanical and biological properties. CHT/PCD sponges showed a high swelling capacity (600%) and moderate lysozyme-induced biodegradation rate in vitro (12% mass loss 21 days). Results of X-ray microtomography showed a high porosity (87%) with interconnected pores. Good cell adhesion and in-growth (colonization) in the sponge were observed by confocal microscopy using pre-osteoblasts and endothelial cells (HUVECs). Biofunctionalization of the sponge by loading VEGF (Vascular endothelial growth factor) showed that the release of VEGF was rapid (burst) during the first two days, then slowed down up to non-detectable by ELISA method after 7 days. The released VEGF during the first two days showed a significant proliferation and pro-migration effect on HUVECs. In conclusion, the developed injectable hydrogel and sponge, based on polyelectrolyte complex between CHT and PCD, showed promising potential for application as scaffold in BTE. Further studies in vivo are expected to fulfill the requirement of clinical application.

**Key Words:** Hydrogel, chitosan, biomaterial, Tissue engineering

### **II.3.1. Gene edition on hydrobiological organisms of aquaculture interest for Perú: Progress and scope**

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**ABSTRACT:** The technique of the Crispr Cas9 System (Clustered Regularly Interspaced Short Palindromic Repeats) Cas9 (Crispr associated protein 9), is currently the most versatile genomic tool created in the history of molecular biology. This system is capable of editing various types of genomes in various hydrobiological organisms. Currently, various hydrobiological organisms among crustaceans, bivalves and freshwater and marine fish. They are being edited worldwide. For Perú, the gene editing targets various salmonids such as rainbow trout, cichlids such as Tilapia, Amazonian fish (Paco, gamitana, paiche, etc.) and marine fish such as sole. In invertebrates, the candidates are the shrimp and the fan shell, among others. The main "editable genes" or are Myostatin to produce "hypermuscularity", several genes associated with resistance to abiotic diseases and stress (thermotolerance, contamination, etc.), prolificacy genes, the tyrosinase gene associated with albinism and Kitlg gene associated with patterns of body color among others. Although the part of gene editing at the nucleotide sequence level is of almost standard application. The step of the embryonic microinjection will depend a lot on the feasibility according to the hydrobiological species, which is a complete biotechnological challenge. Here is a review of its progress and scope for Peruvian fish farming and aquaculture.

**Key Words:** Gene editing, Crispr Cas, hydrobiological, aquaculture, pisciculture.



**II.4.1. Ecology and antimicrobial resistance of *Ralstonia* spp. in the urban water cycle****Félix Pompeyo Ferro Mayhua**

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**Abstract:** *Betaproteobacteria* are ubiquitous in the urban water cycle, with multiple opportunities for interaction with humans. The combination of the physiological and ecological properties of some *Betaproteobacteria* explains their ability to survive in the environment, persist after disinfection of drinking water and sometimes colonize animals, including humans. In this study it was sought to understand if there was an interrelation between resistance to metals and antibiotics and how this could influence the behavior of the strains. The experimental work involved the characterization of strains of the *R. pickettii* and *R. mannitolilytica* species, isolated from hospital sewage, tap water and bottled mineral water, and aimed to identify possible associations of resistance phenotypes to antibiotics and metals and other types of stress, as well as their genetic basis. This study suggested an association between resistance to gentamicin (MIC > 256 mg/L) and increased tolerance to arsenite (MIC = 1.4 mmol/L). In addition, in strains resistant to gentamicin, biofilm formation was stimulated in the presence of aminoglycosides or arsenite. Ultraviolet or hypochlorite disinfection exhibited similar inactivation rates in resistant and susceptible gentamicin strains.

The association between resistance to gentamicin and arsenite was explored in a larger group of *R. pickettii*. The antibiotic and arsenite resistance profiles were screened and selected resistance genetic determinants, including those relating to Integrative and Conjugative Elements (ICEs), plasmids, and genes associated with efflux and arsenic resistance were analyzed. Most of the isolates (32/37) were resistant to gentamicin, beta-lactam and colistin. Moreover, isolates resistant to gentamicin and arsenite showed ICEs and the *arsH* and *acr3* genes, related to the resistance to arsenite. Most of the *R. pickettii* isolates had one or two plasmids with sizes between 77 and 260 kbp. The deduced amino acid sequences of the efflux pump, whose gene amplified in all strains, differed in resistant and susceptible isolates. Although genotypes of resistant strains susceptible to gentamicin differed in a manner consistent with the phenotypes and with the phylogenetic distinction of both groups, it was not possible to find a genetic explanation for the observed phenotype nor for the association of arsenite and gentamicin resistance. However, for all the analyzed genetic elements there was a clear division between both groups.

**Key Words:** Opportunistic pathogens; Stress tolerance; Intrinsic resistance; Acquired resistance.

### II.5.1. Sensory and microbiological characterization of *Allagoptera leucocalyx*

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**ABSTRACT:** The Bolivian Chiquitania is a region of high biodiversity in plant species and uncharacterized<sup>1 2</sup>. The objective of this work is to characterize sensory and biological properties of the *Allagoptera leucocalyx* (Motacuchi) fruit.

Color was determined digitally with high resolution images (CIELAB, ImageJ and Photoshop). The texture was determined by TPA, in a texturometer (conical probe TA17, 24 mm, 1 mm/s, 2 mm deep). The pH was determined (LAQUA potentiometer 9615S). Moisture was determined by weight loss of 10 samples (9 days, 60°C). The antimicrobial activity was determined by turbidimetry (Peptone water) and antibiogram (Mueller-Hinton Agar) against *Shigella*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella*. The bioactive compounds of the fruit were extracted with 70% ethanol (23 g/100 mL) while shaker (18 h), filtered and centrifuged (15 min, 10<sup>4</sup> rpm). It worked with two concentrations (C1, C2). The absorption spectrum of 300-900 nm was carried out.

The values obtained for color were L\*: 41.65; a\*: 23.31, and b\*: 45.07. Chroma (50.8), tone (62.7°) and yellowness index (154.6) were determined, values that indicate medium luminosity and yellowish coloration. pH (4.13, acid) and %humidity (59.86) were determined. The values of firmness, hardness, fracture and elasticity were obtained (2.15 g/s; 42 g; 27 g; 0.3 g), suggesting soft consistency at maturity, confirming with color and moisture in the samples. Antimicrobial effect was found at C2 for *Shigella* in plaque diffusion and turbidimetry. A previous study demonstrated the ineffectiveness of *Allagoptera leucocalyx* leaves against *Escherichia coli*, *Pseudomonas aeruginosa*<sup>3</sup>. The acidic pH of the fruit (4.13) can be a bactericidal factor against *Shigella*, at a pH<4.8 its growth is inhibited<sup>4, 5</sup>. C2 was more effective than C1 due to the better mass transfer<sup>6,7</sup>. The absorption spectrum showed a peak at 664 nm, nitrous group, and also a peak at 435 nm, yellow color range; coinciding with the color determination.

The physicochemical parameters represent the main characteristics for the acceptance of the fruit for its fresh consumption and industrialization. The fruit shows a promising alternative for the control of *Shigella*, whether in the food or pharmaceutical area, creating prospects for agro-industrialization.

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**Key Words:** Microbiology, antimicrobials, motacuchi, fruits

### II.5.2. Studies of tomato lipoyl synthase genes and their potential applications

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**-Introduction:** Lipoic acid (LA) is an eight-carbon molecule with thiol groups at carbons 6 and 8, known for its characteristics as a strong antioxidant. In plants, exogenous application of LA triggers higher resistance to different forms of abiotic stress. However, in vivo it also plays an important role as a cofactor of numerous enzymatic complexes involved in primary metabolism, such as pyruvate dehydrogenase (PDH), and  $\alpha$ -ketoglutarate dehydrogenase (kGDH).

Despite being essential for primary metabolism and thus common in aerobic organisms, LA is synthesised from octanoic acid in an unusual reaction catalysed by a lipoyl synthase enzyme. This reaction involves the addition of 2 thiol groups for which two molecules of S-adenosyl methionine (SAM), and two [4Fe-4S] clusters are needed, coordinated by a key serine residue.

In plants, two PDH complexes can be found, located in mitochondria and plastids, thus requiring the presence of two lipoyl synthases. In tomato, two genes coding potential LIP1 genes have been found: one mitochondrial (SILIP1) and one plastidial (SILIP1p).

**-Methodology:** To verify whether SILIP1 and SILIP1p possess a similar mechanism to previously described lipoyl synthases, site-directed mutagenesis of the key serine residue of both genes was carried out. A complementation assay was performed, transforming the *E. coli* lipoyl synthase lipA mutant, KER176, with the modified LIP1 genes; SILIP1-S359A, SILIP1p-S410A.

**-Results:** The transformed bacterial strains were inoculated in minimal media and a marked decrease in growth was observed, consistent with defects in LA metabolism, as opposed to when grown in complete medium.

**-Conclusions:** We conclude that SILIP1 and SILIP1p encode enzymes whose catalytic mechanism is similar to that of previously described lipoyl synthases. Additionally, as applications of LA can generate abiotic stress-resistant crops, a series of transformation vectors are being generated using the novel Loop Assembly system for overexpressing LIP1 genes in *Arabidopsis thaliana* through means of two different strategies. In one strategy, gene overexpression using a saline stress-inducible promoter is being attempted, and in the other strategy, a CRISPR-based approach using synthetic salt-responsive transcription factors to overexpress endogenous genes is being employed. Funded by Fondecyt 1181198, ANID PIA ACT192073 and Fundación María Ghilardi Venegas.

**Key Words:** Lipoic acid, LIP1, Tomato, *Arabidopsis*

### II.5.3. Functional foods: increased antioxidant capacity in tomato fruits by a higher content of lipoic acid

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**-Introduction:** Functional foods benefit human health. On the other hand, oxidative stress is generated by an increase in reactive oxygen species (ROS) which can damage biomolecules causing sickness. Antioxidants neutralize ROS before they cause cell damage, such that foods enriched in these compounds are considered functional. Among antioxidants, lipoic acid is extremely powerful, as well as being amphipathic, regenerating other antioxidants and functioning in both oxidised and reduced forms. However, lipoic acid is also a cofactor that is associated with several enzymes, including pyruvate dehydrogenase (PDH) and  $\alpha$ -ketoglutarate dehydrogenase (kGDH) complexes, which in turn belong to the TCA cycle. The lipoylation process occurs via two routes, *de novo* synthesis and the salvage of free lipoate. Common to both pathways is lipoyl synthase (LIP1). In order to obtain a tomato fruit with higher antioxidant capability, in the present work, *lipoyl synthase* (*SILIP1*) genes from *Solanum lycopersicum* are being expressed under the control of a fruit-specific promoter.

**-Methodology:** In a binary vector, 800 bp of the fruit specific polygalacturonase (PG) promoter were cloned upstream of *SILIP1* CDS. Tomato leaves (cv Micro-Tom) were transformed using *Agrobacterium tumefaciens* harbouring this vector. Regenerated plants were acclimatised on soil and analysed in order to evaluate the presence of the transgene. The levels of expression of *SILIP1* and of other genes of the lipoylation pathways (*SILIP2* and *SILpIA*) and of the TCA cycle, were measured.

**-Results:** *SILIP1* expression levels rise naturally in untransformed tomato fruits. Of the transformed explants, 10 harbour the transgene and do not present altered vegetative growth. Fruits of 3 lines over-express *SILIP1* transcripts, and 2 of these also over-express the plastidial version of *SILIP1*, *SILIP2* and *SikGDH*. Some lines have increased *SIPG* and *SIPDH* transcript levels, whilst in other lines *SILpIA* transcript levels are higher.

**-Conclusions:** Tomato was successfully transformed with *SILIP1*, causing increases in the expression of associated genes in fruits. The degree of lipoylation is being determined, especially in the lines with an increase in *SIPDH* and *SikGDH* expression, and we expect to obtain a correlation between these results and the levels of lipoic acid and the antioxidant capacity in transgenic tomato fruits. Funded by Fondecyt 1181198 and Fundación María Ghilardi Venegas.

**Key Words:** Lipoic acid, Lipoyl synthase, Antioxidant, Tomato



### II.6.1. Circadian transcriptomics in *Prorocentrum* sp. key dinoflagellate from Algal Blooms of the Eastern South Pacific

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#### ABSTRACT

The identification of the number of circadian transcribed genes in relation to cell division in red tide dinoflagellates has not yet been reported, being endogenous timer biological clocks that allow organisms (from cyanobacteria to humans) to adjust their physiology and behavior through behavioral cycles. and approximate 24-hour physiological functions that function to anticipate daily environmental rhythms. The basic mechanism of the clock consists of a network of transcriptional-translational negative feedback loops that drive the rhythmic expression of genes over a 24-hour period. The objective of this study is to determine the genes transcribed with a circadian expression pattern in the key dinoflagellate isolated from the coasts of Antofagasta during the flowering seasons (August 2018-March 2019) identified as *Prorocentrum* sp. strain ACIZ\_LEM2, evaluating the expression profile of each 4 h for 48 h under conditions of continuous light (LL) and photocycle 14 hrs light / 10 hrs dark (LO). Using RNAseq, the number of circadian transcribed genes will be identified based on the detection of a rhythmic oscillatory expression pattern under LL and LO conditions. The annotation would reveal the specific genes with a circadian expression rhythm related to cell multiplication and other metabolic processes. Identifying the number of circadian transcribed genes in the dinoflagellate *Prorocentrum* sp. and knowing the identity of cell cycle regulators is the first step in designing experiments to determine the molecular events that are responsible for algal blooms in the East South Pacific.

**Key Words:** circadian, cell cycle, RNAseq, transcriptome

**II.7.1. Perception about genetically modified organisms of the citizens of northern Perú 2021**

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**ABSTRACT**

This study presents the results of a survey conducted in northern Perú in the period January-April 2021 in the vicinity of universities in Trujillo, Chimbote and Chiclayo with the purpose of knowing what perception they have about modern biotechnology in the present after the application of genetic engineering especially to food production. An attempt was made to know their knowledge and attitude about the production, consumption and impact on human health of foods obtained through the genetic transfer of new attributes to plants and animals that are used in human nutrition, obtaining the so-called genetically modified organisms. or transgenic. An attempt was made to auscultate the reaction and level of concern that has been generated in the population and to what extent there is awareness of the level of impact on future health and well-being if the production and consumption of transgenic foods is extended nationally and internationally.

It must be recognized that, although genetically modified foods are a major source of controversy worldwide, foods that contain genetically modified components are found in virtually all markets and tables across the country and abroad. It is impossible to know the exact figures for the consumption of these products. It is argued that in the USA, most estimates suggest that between 60% and 70% of processed foods on US shelves include at least one fragment of a genetically modified crop (GEO-PIE, 2003). This prevalence is due to the fact that most of the soybeans and rapeseed (canola) and one third of the corn harvested in the United States and Canada in 2002 were transgenic varieties (GEO-PIE, 2003). Additionally, these crops are the source of some of the most common ingredients used in food production, and because GM varieties of corn, soybeans, and canola are often mixed with ordinary varieties, incorporating at least small amounts of GM ingredients in many processed foods is a reality and a virtually unavoidable problem. For example, many sweetened processed foods such as sodas and baked goods often contain high fructose corn syrup obtained from a silo storage system in which genetically modified corn is not tracked or treated differently than varieties. non-transgenic. Currently, there is little diversity among the transgenic products available; corn, soybeans,. The population tends to differ in some aspects regarding their perception and attitude towards GMOs in consideration of sex, age group, educational level and occupation. For this reason, it is necessary to implement awareness, education and orientation campaigns on the benefits, limitations and still unknown situations regarding GMOs in order to achieve their application in an efficient, objective and profitable way.

### III.1.1. Water-Holding Capacity and Antioxidant Activity of Bovine Skin Gelatin Hydrolysates as Potential Polyphosphate Substitutes

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- **Introduction:** Currently, there is a growing demand for new healthy foods. Additives such as polyphosphates are used in the meat industry to improve water retention and its concomitant effect on its products' juiciness, texture, and final weight. On the other hand, it has been reported that excessive amounts of these additives in the diet can be harmful to health. In this sense, gelatin as a source of collagen is a protein with excellent biological compatibility and low allergenicity that can be used as an additive in the meat industry. In this work, the addition of bovine skin gelatin hydrolysates (BSGHs) on the water-holding capacity (WHC) and antioxidant activity in a chicken meat model was studied.

- **Methodology:** The bovine skin gelatin was hydrolyzed with subtilisin at two levels of degree of hydrolysis (DH) (BSGH1 and BSGH2). The samples were prepared with ground chicken meat and the different hydrolysates at 0-5% (w/w) levels. The WHC was measured as cooking loss, subjecting the samples to heat treatment and quantifying the water lost during this process. Also, the solubility, antioxidant activity of BSGHs and their effect on the texture and color of the meat samples were measured.

- **Results:** The results showed that both hydrolysates (BSGH1 with 6.57% DH and BSGH2 with 13.14% DH at 3 and 2.5% (w/w) concentration, respectively) improved the WHC in the meat matrix and had behaviors equal to those of the ingredient commercial sodium tripolyphosphate (STPP) at its maximum allowed limit (0.5% (w/w)). Furthermore, BSGH2 reached the same firmness as STPP in its maximum limit allowed in the food industry when it was applied at 5% (w/w) in the meat matrix. BSGH1 and BSGH2 reached a color difference similar to STPP in its maximum allowed limit when applied at a concentration of 2% (w/w). On the other hand, it was found that these hydrolysates reached the same antioxidant activity as STPP.

- **Conclusions:** In conclusion, we found that BSGHs can be an efficient replacement for STPP to achieve equal or better water retention in thermally processed chicken meat. These reached the same firmness, color difference and antioxidant activity as the commercial ingredient STPP at its maximum limit allowed in the food industry.

**Key Words:** water retention, antioxidant activity, hydrolyzed gelatin, polyphosphates

### III.2.1. Evaluation of the Physicochemical Properties of Recycled Edible Oils as a source for obtaining Bioglycerin and Biodiesel through Biological Processes, in the city of Arequipa.

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- **Introduction:** In the food industry, the number of edible oils used in mainly frying processes has now reached considerable levels as a by-product in various food processing facilities. In such a way, that once the collection and storage points of the Recycled Edible Oils have been identified, they are characterized in terms of their Physicochemical Properties, through Official Food Analysis Methods, which allows to recognize the characteristics of such waste from the Food Industry, for subsequent processing in obtaining Bioglycerin and Biodiesel, by the action of Biological Agents.

- **Methodology:** Through stratified sampling, a total of 50 sampling points has been considered for the collection of recycled edible oil. Regarding the collection and collection procedure, recycled edible oil has been stored and transported at an average temperature of 23 ° C, in polyethylene terephthalate (PET) containers, to the Research and Services Laboratories (LABINSERV) of the University National of San Agustín de Arequipa (UNSA).

- **Results:** The results obtained are shown: Humidity 0.09%, Ash 0.01%, Density 0.9171 g / mL, Acid Index 3.52% Ac. Oleic, Refractive Index 1.4744, Saponification Index 196.81 mg KOH, Peroxide Index 10.15 meq / 1000 g, Impurities 0.14%, Unsaponifiable 0.13%, Oxidized Acids 0.89%, Flash Point 317.00 ° C, Determination of Soap > 0.001%, Phosphorous 81.65 ppm, Iron 0.66 ppm, Lead 1.45 ppm, Copper 0.92 ppm

- **Conclusions:** Action plans for the collection and stockpiling of recycled edible oils from both government and private entities are relevant for the proper management of these viscous fluids, which involve training and the creation of a recycling culture in the population involved in their handling, both domestically and industrially, in the city of Arequipa.

The recorded values of the methods and laboratory tests in the analysis of characterization of recycled edible oils show differences with previous publications regarding edible oils, it is important to consider that the characterization of recycled edible oils allows information to interact for proposals of their processing for the benefit of maintain the environmental sustainability of cities.

**Key Words:** Bioglycerin, Biodiesel, Recycled Edible Oils.



**III.2.2. Elaboration of compost from household organic waste and water lentil (*Lemna spp.*) with application of effective microorganisms (EM)**

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**ABSTRACT**

In order to make compost from domestic organic waste and Duckweed (*Lemna spp.*) Applying effective microorganisms (EM) at three doses (0, 100 and 200 mL), the decomposition time and granulometry, temperature, pH were evaluated. , nitrogen, phosphorus, potassium and sodium, with the objectives of: **a)** evaluate the decomposition time of the organic residue and *Lemna spp.* **b)** evaluate the temperature and pH, **c)** analyze the concentration of nitrogen, phosphorus, potassium and sodium in the compost. Analysis of variance and multiple rank test were used. The results show that the decomposition time at 75 days was duckweed; 50 days solid residues both with 200 mL of EM. The granulometry indicates 75 to 90% of granules with diameters <1.5 mm in both compost. The temperature at 26.56 °C for duckweed and 27.48 for organic waste, similar to each other ( $P > 0.05$ ); with 200 mL of EM. Duckweed pH 6.5, organic residues 6.4 similar to each other ( $P > 0.05$ ). For the doses of EM, neutral pH and similar to each other ( $P > 0.05$ ), nitrogen, phosphorus and potassium, no statistical difference was found ( $P > 0.05$ ), for total sodium duckweed presents a higher value than organic residues. Nitrogen 0.33% at 200 ml of ME, statistically higher than the rest of the doses ( $P < 0.05$ ). Phosphorous with EM doses: 100 mL presented 41.075%, 200 mL at 43.72%, being greater than the control and similar to each other ( $P < 0.05$ ), the control with 22.305%. For potassium with EM doses: 200 mL presented 0.685%, 100 mL with 0.595% and the control 0.5%, with no statistical difference between them ( $P > 0.05$ ). Sodium presents similar averages 662.5 and 725.0 respectively, 100 and 200 mL of EM. Resulting in that domestic organic waste tends to decompose more quickly than duckweed and also incorporating the essential elements for autotrophic nutrition.

**Key Words:** Compost, effective, granulometry, microorganisms.

### III.2.3. De novo elucidation of the bacterial catabolic pathway of the fluorinated pesticide epoxiconazole

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#### ABSTRACT

Epoxiconazole (EPO) is a popular fluorinated pesticide with significant environmental impacts, mostly due to its persistence and broad non-target toxicity. Until recently, microorganisms were thought to have a minor role in its environmental fate due to its recognized recalcitrance. Yet, we recently showed that an enriched bacterial consortium was able to consume and defluorinate EPO in a wide concentration range. To characterize the microbial processes underlying this unusual catabolic proficiency, this work aimed at elucidating the biochemical pathway involved in the biodegradation of EPO. An integrated multi-omics approach was implemented by combining metagenomics, metaproteomics and metabolomics data to resolve the EPO biodegradation pathway at the genotype and phenotype levels. An EPO biodegradation assay was conducted using the high-performing consortium as inoculum, and sodium acetate as a secondary carbon source, for a total period of 28 days. Cultures supplemented only with sodium acetate were used as controls. All cultures were sampled for multi-omics analysis at days 0 and 14, whereas for defluorination analysis sampling was done also after 28 days. The consortium was able to completely remove EPO from the culture medium and to defluorinate ca. 70% of this pesticide. Based on the EPO subproducts tentatively identified by LC-MS, the epoxy bridge seems to be the primary site of microbial attack, followed by the dissociation of the triazole moiety from the diaryl backbone. Given the substantial defluorination detected, it is possible that EPO biodegradation also involved dearomatization reactions. Several catabolic enzymes capable of catalysing the forementioned reactions were found to be expressed in the degrading cultures, including various lyases, oxidoreductases and hydrolases. Various other relevant proteins were also identified, including proteins related with pesticide uptake, chemotaxis and homeostasis regulation. The existence of a significant fraction of silent genes coding for other catabolically relevant proteins suggests that this consortium possesses several other potential catabolic strategies to achieve the biodegradation of EPO. This work details for the first time the bacterial catabolic pathway of EPO, providing highly relevant data not only to better understand the environmental fate of this persistent pesticide, but also for the design of novel bioremediation technologies targeting this compound.

**Key Words:** metabolic pathway; pesticide biodegradation; multi-omics; defluorination

**III.2.4. The Biotechnological potential of Cyanobacteria and Microalgae Bioactive Compounds in combating the signs of Skin-aging**

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**ABSTRACT**

As the largest organ in the human body, skin acts as a physicochemical barrier, offering protection against harmful environmental stressors, such as chemicals, pathogens, cold, heat and ultraviolet radiation. Nonetheless, skins prominence goes even further, with a significant psychosocial role, related to health and in an increasingly aging population. Prompted by the consumers' concern regarding skincare, the cosmetic industry has been developing new formulas capable of lessening the most visible signs of aging, including reduction in skin density and elasticity, increased wrinkles formation, and hyperpigmentation. Allied to skincare is the rising importance set on using natural products, with less side effects, and obtained by sustainable and less environmental impacting methods. As sources of natural ingredients for skin formulations, cyanobacteria and microalgae are adding importance, due to their capacity to biosynthesize secondary bioactive metabolites with potentialities to be explored in the field of anti-aging. In this review, we present an overview on the potential of cyanobacteria and microalgae compounds to overcome skin-aging, essentially by exploring their effects on target enzymes, such as the metalloproteinases collagenase, elastase, gelatinase and hyaluronidase, and other involved in the control of the pigmentation process.

**Key Words:** matrix metalloproteinases; collagenase; elastase; hyperpigmentation.

### III.2.5. Evaluation of the deproteinization of shrimp waste (*Litopenaeus vannamei*) through the use of proteolytic bacteria and different operating conditions.

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**-Introduction:** Shrimp production in Perú was 33,819.49 MT in 2019, representing 57.7% of aquaculture exports (1,2). The tails of these are commercialized, remaining between 60% and 80% of the weight as waste, which is a source of products of commercial interest (3) that could be recovered through several processes, being the first one the deproteinization process (DP). Traditional methods use inorganic bases at high concentrations and temperatures that can generate corrosive and toxic substances. It has also been reported that DP can occur with enzymes and microorganisms (4-6). The present work aimed to deproteinize shrimp wastes (SW) using native proteolytic bacteria and evaluate deproteinization process time.

**-Methodology:** The SW were desanded, washed, dried and crushed generating a uniform powder. Two strains (C1 and C2) of the genus *Shewanella* spp., were studied and selected for their high proteolytic capacity (7). DP assays were performed under a factorial design 22 which measured the effects of process temperature and time on the deproteinization percentage (% DP). The process conditions were 4 and 8 hours, 25 and 35 °C, 6 mL of inoculum at 108 CFU/g, 170 rpm and 0.5 vvm aeration. All assays had 18 ± 1g of SW and 126 mL of 75% seawater. The SW and products obtained were characterized by measuring the content of moisture, ash, fat, protein and degree of deacetylation (8-12).

**-Results:** The SW contained initially 5.3% moisture, 51.8% ash, 1.3% fat and 10% protein. SW protein was reduced in all trials by more than 30% with the strains under study. The strain with the highest DP capacity was C1, which reached 44.1% DP in 8 hours at 35 °C and C2 reached 35.2% DP in 8 hours at 20 °C. The interaction temperature-time had an effect on the behaviour of C2 but not on C1 despite being species of the same genus. The behaviour of strain C1 was influenced by the single effects of process temperature and time.

**-Conclusions:** Strains C1 and C2 managed to reduce protein above 35% in 8 hours; C1 reached 44% of DP being the highest value obtained. The interaction temperature-time had a significant effect on C2 and the singular effects of process temperature and time influenced C1.

**Key Words:** Deproteinization, shrimp waste, process time

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### III.2.6. Natural cosmetics based on cyanobacteria: searching for safe and profitable formulations

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#### Abstract

The use of natural products in skin care formulations gained has interest as a concern for modern societies. The undesirable side effects of synthetic compounds, as well as the associated environmental hazards, have driven investigation on photosynthetic organisms as sustainable sources of effective and environmentally friendly ingredients. The use of natural extracts in cosmetics has been highlighted and, along with plants and algae, cyanobacteria have come into focus. Due to their low culture demands, high grow rates and ability to produce a wide variability of bioactive metabolites, cyanobacteria emerged as an economic and sustainable base for the cosmetic industry. In this study, we evaluated the potential of ethanol extracts of picocyanobacteria strains of the genera *Cyanobium* and *Synechocystis* and filamentous strains of the genera *Nodosilinea*, *Phormidium* and *Tychonema* for skin applications, with focus in the field of anti-aging. The extracts were analyzed for their pigment profile, phenolic content, antioxidant potential, cytotoxicity against keratinocytes (HaCat), fibroblasts (3T3L1), endothelial cells (hCMEC/D3) and capacity to inhibit hyaluronidase (HAase). The total carotenoid content ranged from 118.69 to 383.89  $\mu\text{g g}^{-1}$  of dry biomass, and the total phenolic content from 1.07 to 2.45 mg GAE  $\text{g}^{-1}$ . Identified carotenoids consisted of zeaxanthin, lutein, canthaxanthin, echinenone and  $\beta$ -carotene, with zeaxanthin and lutein being the most representative (49.82 and 79.08  $\mu\text{g g}^{-1}$ , respectively). The highest antioxidant potential was found for *Phormidium* sp. LEGE 05292 and *Tychonema* sp. LEGE 07196 for superoxide anion radical ( $\text{O}_2^{\cdot-}$ ) scavenging ( $\text{IC}_{50}$  of 822.70 and 924  $\mu\text{g mL}^{-1}$ , respectively). Low or no cytotoxicity was registered. Regarding HAase inhibition, *Tychonema* sp. LEGE 07196 and *Cyanobium* sp. LEGE 07175 showed the best  $\text{IC}_{50}$  (182.74 and 208.36  $\mu\text{g mL}^{-1}$ , respectively). In addition, an increase in fibroblast proliferation was registered with these same strains. From this work, the ethanol extracts of the species *Tychonema* sp. and *Cyanobium* sp. are particularly interesting for their potential application in anti-aging formulations, once they stimulated fibroblast proliferation and inhibit hyaluronic acid digestion.

**Key Words:** carotenoids; phenolic content; anti-aging; hyaluronidase

### III.2.7. Biosorption of Zn(II) from seawater solution by microalgal biomass of *Tetraselmis marina* AC16-MESO

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**Background:** Biosorption is defined as a physicochemical process in which substances are removed from solution by a biological material (live or dead) via adsorption processes governed by mechanisms such as surface complexation, ion exchange, precipitation. Therefore, this study aimed to evaluate the adsorption of Zn<sup>2+</sup> in seawater by microalgal biomass of *Tetraselmis marina* AC16-MESO “in vivo” and “not alive” at different concentrations of Zn<sup>2+</sup> (0, 5, 10 and 20 mg/L) at 72 h using the Langmuir isotherms, evaluating the autofluorescence from microalgae.

**Results:** The maximum adsorption of Zn<sup>2+</sup> by the Langmuir model using the Q<sub>max</sub> parameter in the living microalgal biomass (Q<sub>max</sub> = 0.03051 mg/g) was greater than the Non living microalgal biomass of *T. marina* AC16-MESO (Q<sub>max</sub> = 0.02297 mg/g). A decrease in fluorescence was observed in cells from *T. marina* AC16-MESO, in the following order: (0 < 20 < 5 < 10) mg/L Zn<sup>2+</sup>.

**Conclusions:** Zn<sup>2+</sup> was more quickly adsorbed by living cells from *T. marina* AC16-MESO than that by Non living microalgal biomass, with a decrease in photosystem II activities were observed from 0 to 20 mg/L Zn<sup>2+</sup> in living cells..

**Key Words:** autofluorescence, adsorption, microalgal, Langmuir.

### III.2.8. Characterization of cyanotoxins and cyanobacteria secondary metabolites from alqueva - first results

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**-Introduction:** In Portugal, the Alqueva freshwater system represent one of the most important economic drivers in Alentejo, by providing freshwater for Agriculture, in a region heavily affected by water shortages.

In a climate change context, freshwater cyanobacteria possess natural advantages, benefiting from eutrophication conditions, e.g. elevated nutrients, high CO<sub>2</sub> and high temperatures. Some cyanobacteria are responsible for the production of cyanotoxins. Due to their potent action, the release of these toxins can lead to severe consequences in other organisms, greatly affecting the water quality. Although regulatory limits were redefined in 2020 by World Health Organization for drinking and recreational waters, no limits have been defined for waters used in crops irrigation. Several studies raise concerns about the presence of toxic cyanobacteria in reservoir waters and its recurrent use in agriculture, which can lead to a significant contamination of the soils and crops.

This work intended to characterize the presence of different cyanotoxins (and other secondary metabolites) in waters from the Alqueva system. The data will be used to assess the water quality and evaluate the risks associated with the recurrent use of these waters in agriculture.

**-Methodology:** For that, a total of 28 water samples were collected in a campaign carried out in the summer of 2020 (July, August and September) in the Alqueva system. The samples were processed in the laboratory and analyzed for the presence of cyanotoxins employing Liquid Chromatography and Mass Spectrometry (LC-MS).

**-Results:** Preliminary results revealed the presence of MC-LR in some water reservoirs. The highest concentration found was 0,10 µg MC-LR /L in S. Pedro reservoir, in July.

**-Conclusions:** Despite the absence of toxin concentrations above the regulatory limit of 1 µg/L (in drinking waters), this monitoring exercise is necessary to follow the levels of different cyanotoxins, given the recurrent presence and proliferation of toxic cyanobacteria in the Alqueva system.

**Key Words:** Cyanotoxins, MC-LR, Alqueva, LC-MS.

### III.2.9. Identification and characterization of bacteria with the biodegradation capacity of low-density polyethylene and polyethylene terephthalate isolated from El Tragadero hot spring, Baños del Inca-Cajamarca.

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#### **-Introduction:**

In Perú, almost 3 million plastic bags are accumulated each year (MINAM, 2019), for that reason, some strategies such as biological treatments have been started to solve this problem. Among the organisms involved in these processes are thermophilic bacteria (optimal growth temperature  $\geq 45$  °C) whose enzymes (hydrolases) can interact with plastics. The great enzymatic activity at high temperatures offers a sustainable alternative to eliminate these polymers from the environment. Current research, financed by FONDECYT (contract N° 395-2019), has the aim to identify and characterize thermophilic bacteria with plastic biodegradation capacity from a hot spring in the Cajamarca region.

#### **-Methodology:**

The study was carried out in “El Tragadero” hot spring ( $\sim 70$  °C), located in Cajamarca. Isolation of bacteria was performed with two types of enrichments, *ex-situ* (at the laboratory) and *in-situ* (at the field). To test the use of LDPE (low-density polyethylene) and PET (terephthalate of polyethylene) by the isolates, biodegradation assays were done, in which the percentage weight loss (%PP), the rate constant of polymer reduction ( $K$ ), and the half-life ( $t_{1/2}$ ) were calculated. The isolates that decreased the polymer weight were considered as cultures with LDPE/PET biodegradation capacity and will be microbiological and molecular characterized.

#### **-Results:**

17 isolates were obtained from the *ex-situ* enrichment and 37 from the *in-situ* enrichment. The biodegradation assays with LDPE showed that 10 isolates achieved a %PP between 0.1 and 3.9 %, being 16E and 13L the cultures with the highest reduction ( $3.9 \pm 1.9$  % y  $1.3 \pm 1.1$  %, respectively). Culture 16E, showed a  $K$  value of  $0.00044 \text{ g day}^{-1}$  and a  $t_{1/2}$  value of  $5.7 \pm 2.9$  years. Regarding biodegradation assays with PET, 5 isolates achieved a %PP less than 1%. In the non-inoculated controls, only those with LDPE did not decrease their weight. The microbiological and molecular characterization is in process.

#### **-Conclusions:**

The thermophilic bacteria isolated from El Tragadero hot spring (Cajamarca) have the biodegradation capacity of plastic polymers such as LDPE/PET. Current research can contribute to developing an efficient and sustainable alternative for the elimination of plastics both in Cajamarca and throughout the country.

**Key Words:** Thermophilic bacteria, LDPE, PET, biodegradation.



**III.2.10. Cyanobacterial bioactive nucleosides with antifouling properties**

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**ABSTRACT**

Marine biofouling represents an important issue worldwide to marine industries, and its prevention causes environmental problems due to the antifouling biocidal agents currently in use. Natural products have the potential to provide alternative solutions for antifouling purposes, that are effective and ecologically compatible. Cyanobacteria are a promising source of bioactive compounds, including antifouling bioactive compounds, given their diversity of secondary metabolites produced and previously documented. This study aims to explore the metabolic diversity of a range of cyanobacterial strains from LEGE Culture Collection in search for environmentally friendly bioactive compounds for antifouling purposes. A library of fractions, derived from methanolic extracts, belonging to different cyanobacterial strains, was tested towards the settlement of a prominent macrofouling organism (*Mytilus galloprovincialis* larvae). Promising fractions were submitted to a bioassay guided sub-fractioning that led to the isolation of two compounds. Their structure elucidation was determined by spectroscopic techniques such as 1D and 2D nuclear magnetic resonance and by mass spectrometry. Analysis of the data indicated the compounds to belong to the nucleoside class. Antifouling bioactivity was assessed on other levels of biological organization such as microfouling organisms (marine bacteria and microalgal biofilms). Future aspects to be considered are the compounds' potential environmental toxicity to the marine environment and the identification of their antifouling molecular targets responsible for the bioactivity in target species.

**Key Words:** Natural products, Cyanobacteria, Antifouling

### III.2.11. Influence of the photoperiod and dilution in industrial wastewater treatment through a microalgae consortium.

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**-Introduction:** The treatment of wastewater is one of the most important challenges today, being tannery wastewater one of the most complex to be treated due to its high load of nutrients and toxic compounds. In the present study, the influence of the photoperiod and the concentration of chromium-free tannery wastewater with a pre-treatment in biomass production and the elimination or reduction of nutrients through a consortium of native microalgae was evaluated.

**-Methods:** During the experimental development, an initial inoculum concentration of  $1 \times 10^5$  cells/mL was used, using 30% of the total volume (200 mL) in all treatments. The microalgae were cultivated in a pre-treated tannery effluent, due to the high presence of suspended solids, the initial chemical characterization of the pre-treated effluent being the following: 1994 mg/L COD, 4.92 mg/L P-PO<sub>4</sub>, 55 mg/L N-NH<sub>3</sub>. Bioremediation was evaluated in duplicate in three different dilutions: 100% wastewater (100% TW), 75% tannery wastewater and 25% distilled water (75% TW) and 50% tannery wastewater and 50% distilled water (50% TW). two photoperiods of 24 h light and 16 h light/8 h dark, which were carried out in 200 ml flasks with shaking system for a period of 10 days.

**-Results:** The results showed that a greater removal was obtained on day 10 with respect to the COD, where a removal of 50.15% was achieved in 50% TW and 38.28% in 75% TW with a photoperiod of 16: 8 compared to the photoperiod of 24: 0 where a removal of 42.9% and 33.13 was obtained in 50% TW and 75% TW, respectively. Regarding nitrogen removal, the results show a removal of 49.09% and 43.03% in 75% TW in the 16: 8 and 24: 0 photoperiods, respectively on day 10. On the other hand, the removal of phosphorus in all the treatments (50%, 75% and 100%) had a removal greater than 90%.

**-Conclusions:** Finally, the microalgae consortium showed a high growth performance in the treatment of tannery effluents under conditions and with a 16: 8 photoperiod. Phosphorus as well as nitrogen play an important role for the synthesis of molecules important for cell growth. Since it is in short supply in the environment, it limits cell growth, causing the elimination of other compounds to be affected. In this sense, the microalgae bacteria consortium has shown to have a high consumption rate, having elimination percentages of over 80% on day 5 of culture. Microalgae, due to their great ability to remove nutrients from aqueous media, can be used as an ecological alternative for treating wastewater from tanneries.

**-KEY WORDS:** Microalgae, photoperiod, effluents, tanneries.

### III.2.12. Molecular identification and evaluation of resistance to heavy metals by native bacteria isolated from tannery wastewater.

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**-Introduction:** Chromium (VI) is a toxic metal used in large quantities in the leather industry. In developing countries, it is common to discard it without prior treatment, causing negative impacts on the environment and human health. In the present study, it was possible to isolate and identify three native bacteria that reduce Cr (VI) obtained from the waste lagoons of the Arequipa Industrial Park formed by the effluents of untreated tanneries, as an alternative to reduce Cr (VI).

**-Methodology:** The experimental tests consisted of sampling, isolation, morphological characterization, measurements of bacteria growth by optical density at 600 nm, evaluation of the maximum tolerance concentration (MTC) (0-2400 mg L<sup>-1</sup>) at 24 h, Molecular identification using the 16S rDNA gene and determination of the bacterial reduction capacity against Cr (VI) at different concentrations (30, 60 and 100 mg L<sup>-1</sup>) for 36 h. Additionally, tolerance to other heavy metals (Cu<sup>+2</sup>, Zn<sup>+2</sup>, Cd<sup>+2</sup>, Pb<sup>+2</sup>, As<sup>+2</sup>) was evaluated.

**-Results:** As results, in the isolation 6.70 x10<sup>4</sup> CFU mL<sup>-1</sup> and 1.34 x10<sup>5</sup> CFU mL<sup>-1</sup> were obtained at 24 and 48 h respectively after the plate was inoculated with the mother sample at a dilution of 10<sup>-2</sup>, isolating 10 bacteria, of which the *P. mirabilis* bacteria showed tolerance to 2000 mg L<sup>-1</sup> of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, while *H. campaniensis* and *B. pumilus* were able to tolerate 2400 mg L<sup>-1</sup> of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>; Likewise, these bacteria showed a Cr (VI) reduction capacity of 99.00%, 98.68% and 98.28% at 30 mg L<sup>-1</sup>; 89.20%, 36.59%, and 46.27% at 60 mg L<sup>-1</sup>; and 78.05%, 29.46% and 35.26% at 100 mg L<sup>-1</sup> respectively. Of all the bacterial strains, *P. mirabilis* showed a tolerance to all heavy metals, except for Zn<sup>+2</sup>, however, for the strain *H. campaniensis* a minimum tolerance was observed for all heavy metals and *B. pumilus* was affected by Cu<sup>+2</sup> and Zn<sup>+2</sup>.

**-Conclusions:** Therefore, the bacteria isolated in the present investigation can be an eco-friendly alternative for the remediation of Cr (VI) from contaminated environments.

**-Key words:** Optical Density, Maximum tolerance concentration, Remediation, bacteria.

### III.3.1. Reuse of inoculum from three serial fermentations of ale craft beer on fermentative parameters, volatile compounds and organoleptic properties in final product

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**-Introduction:** World markets has been experiencing a new social trend that motivates consumers to seek more natural products, generating a growing niche for craft beers. In order to reduce costs, a very common practice in craft breweries is to collect the yeast from the fermenter and reuse the recovered biomase in a new, freshly brewed wort. There is evidence from transcriptional analysis that warns of the wear suffered by the inoculum as result of the stressfull conditions of the beer fermentation process, howerever, due to the lack of studies focused on parameters of interest, many producers consider that this practice can be perform indiscriminately without repercussions on the final product

**-Methodology:** Three serial fermentations were carried out in a 50-liter conical fermenter. The first fermentation started with virgin inoculum. After the conditioning process, the biomass was recovered and reused to start the second serial fermentation, repeating the same process for the third fermentation. Fermentative parameters of each serial fermentation, volatile compounds by Gas Chromatography and organoleptic properties of the final product through a sensory panel with 30 panelists were evaluated

**-Results:** A progressive deterioration of fermentative parameters was evidenced in each serial fermentation, increasing fermentation times (54, 68 and 76 hours respectively) and doubling time (8.07, 8.92 and 11.42 hours respectively), and cell viability and specific growth rate (0.0859, 0.0777 and 0.0607 h<sup>-1</sup> respectively). Chromatographic analyzes did not identify intrusive aroma-active compounds. In the sensory panel, the final product of the first fermentation was assessed with the best scores on a 9-point scale for the hedonic scale (average score of 8.0 of acceptability with low dispersion among panelists) and intensity scale (average scores with  $p < 0.05$  in bitterness 5.7, body 6.0 and flavor 7.0), followed decreasingly by the final products of the second and third fermentation

**-Conclusions:** The results obtained suggest that there are significant disadvantages in the reuse of cell inoculum from the second serial fermentation, evidencing disturbances in the fermentative parameters and organoleptic characteristics of the final product that can be percieved by consumers

**Key Words:** beer, reuse, inoculum, serial



### III.4.1. Copper bioleaching using a native microbial consortium with molecular characterization from ancient mining tailings from the Arequipa region

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**-Introduction:** Mining environmental liabilities in Perú are considerable and continue to cause damage to the ground, water and landscapes, due to the use of inefficient traditional methods and the lack of interest from regulatory bodies. To the south east of Arequipa there are inactive copper miners with tailings dumps that are scattered in an area that has been called "Archaeological Landscape", these same environments present virgin soils that have a microbiota for bioremediation studies. That is why the main objective of this study is the recovery of copper in bioleaching processes using isolated microbial consortia from mining tailings, for which the sample will be collected from contaminated soil in a Yarabamba-Arequipa mine, crushed, pulverized and sift, for the characterization of its components.

**-Objective:** Evaluate copper recovery in bioleaching processes using isolated microbial consortia from mining tailings from an old mine in the Arequipa region.

**-Methodology:** It will be evaluated with two treatments, one will be a native microbial consortium (CMN) and the other adapted microbial consortium (CMA), the latter supplemented with  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , where the concentrations of ferrous sulfate will be decreased as shown (50%, 25%, 12.5%) until they are able to adapt without the compound for two months for the bioleaching process, both series will be subjected to horizontal agitation for 30 days. The treatments will be exposed to the mineral for 30 days, will be monitored and cell counts will be made every seven days. Subsequently, DNA extraction and metagenomics will be carried out through "new generation sequencing", finally for statistical analysis comparisons will be made between the abundance of the species obtained between the treatments and the parameters measured: Copper concentration, Potential oxide reduction, pH. The data will be standardized by means of the analysis of variance of a factor (ANOVA), with a level of significance  $p \leq 0.05$ .

**-Results:** Establish or formulate an adequate methodology to evaluate copper recovery in bioleaching processes using microbial consortia isolated from mining tailings from an old mine in the Arequipa region.

**-Conclusions:** The proposed objectives will be met as the indicated methodology (experimental plan) is fulfilled.

**-Key Words:** Metagenomics, bioleaching, mining tailings, copper.

### III.4.2. Application of Extremely Acidophilic Microorganisms for the extraction of $\text{Cu}^{+2}$ from Printed Circuit Boards (PCB) by Mycohydrometallurgy.

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**-Introduction:** The current electronics industry has a high demand for copper (Cu) in the manufacture of printed circuit boards or PCB (printed circuit board) for the assembly of intelligent electronic devices (Smartphone, Tablet or others) and that as a result of mass consumption generates a large amount of electronic waste (PCB) with a high content of copper and metals.

**-Methodology:** In the research, isolates of fungi and bacteria were made from extremely acid leachate cultures (9K liquid medium - pH 1.8) from the biomining laboratory of the University of Antofagasta (Chile), which were identified by optical and confocal microscopy. The percentage of copper ( $\text{Cu}^{+2}$ ) oxidized and recovered in 9K liquid solution was evaluated by means of treatments (static and in aeration system) by mycohydrometallurgical bioleaching, using the bacteria consortiums (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferridurans*, *Leptospirillum ferrooxidans*) and fungi (*Acidithiobacillus acidophilus*). The bioleaching processes were carried out with the PCB plates (6%) sieved in 60 mesh with a particle size of 0.250mm, in a static system and in aeration in a 2L kitasate flask at 28 ° C for 40 days, using 4 treatments (T1 = Negative control; T2 = Bacteria; T3 = Bacteria + Fungus and T4 = Fungus). Cupric ion was quantified using the Atomic Absorption equipment to evaluate the concentration of oxidized copper ( $\text{Cu}^{+2}$ ). Statistical analyzes of the different treatments and significant differences were carried out using ANOVA and Tukey tests.

**-Results:** A copper recovery percentage ( $\text{Cu}^{+2}$ ) of 35% (w/v) was obtained by bioleaching (bacteria) and 60% by mycohydrometallurgy (bacteria and fungi) by treatment with aeration.

**-Conclusions:** Microhydrometallurgy and bioleaching can be a method to obtain cupric solutions ( $\text{Cu}^{+2}$ ) in the oxidation treatments of PCB boards from electronic waste. Copper solutions ( $\text{Cu}^{+2}$ ) could be used in cleaning and disinfection formulations for the control of pathogens (bacteria, viruses, fungi) on hospital or domestic surfaces and areas.

**Key Words:** Bioleaching, Mycohydrometallurgy, Copper ( $\text{Cu}^{+2}$ ), PCB plates.

**III.4.3. Determination of the transcriptomic response to heavy metals in optional bacteria from the spill of a mining tailer in Orcopampa-Castilla, Arequipa, Perú**

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**ABSTRACT**

One of the major pollutants of soils is due to spills from mining extraction activities, to mitigate such events in the world, organisms capable of precipitating and immobilizing toxic heavy metals, facultative bacteria who represent a community Bacterial bacterial important within microbial biodiversity, since by studying their transcriptomic responses the genes that participate in both positive and negative regulation prior to the study of RNA-seq. That is why the main objective of the present study is the study of a facultative bacteria from the soil impacted by mining in Orcopampa-Castilla. For which the sample will be collected from the contaminated soil, it will be installed in a Winogradsky column. After thirty days at room temperature, the facultative bacteria will be isolated for molecular identification. Subsequently, it will be exposed to the highest heavy metal in the contaminated environment, at different concentrations, and in the same way, spending thirty days at room temperature, the extraction and sequencing of the RNA seq Transcriptome will be carried out.

### III.5.1. Bacterial contamination in inert and live surfaces of meat vendors in Puno markets

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#### ABSTRACT

The study was to evaluate the contamination of *Escherichia coli* and *Staphylococcus aureus* in the inert surfaces of the stalls (utensils) and live surfaces (hands) of red meat vendors in the Central, Unión and Dignidad - Puno Markets. Fifty samples were used, collected by means of the peptone solution swab technique and hand washing in vacutainer tubes; These were processed in the Microbiology laboratory - FMVZ- UNA Puno, using the Standard Plate Count method. The indicators expressed positive / negative and / or colony forming units (CFU/cm<sup>2</sup>), on salty mannitol agar (M-S) and methylene blue eosin (EMB); data were analyzed using Chi square test. The results of contamination with *E. coli*, in inert surfaces of the utensils used by the Central Market vendors, was 90% (332 CFU/cm<sup>2</sup>), which exceeds the permissible limit. In Unión y Dignidad, 100% (118 CFU/cm<sup>2</sup>) of inert surface exceeded what is permissible according to standard No. 461-2007/MINSA. In living surfaces from the hands of Central Market vendors, 80% (552 CFU/hand), and Unión y Dignidad 60% (296 CFU/hand) exceed what is permissible. Contamination with *Staphylococcus aureus* in inert surfaces of the Central Market were not permissible 100% (136 CFU/cm<sup>2</sup>), and in Unión and Dignidad 95% (122 CFU/cm<sup>2</sup>). The living surfaces of the Central Market vendors were not permissible with 40% (285 CFU/hand), and in the Unión y Dignidad market 60% (185 CFU/hand) also not permissible. The largest proportion of surfaces exceed the permissible limits for contamination.

**Key Words:** *Escherichia coli*, *Staphylococcus aureus*, inert surfaces, live

### III.5.2. Species of *Trichoderma* producers of $\beta$ -galactosidase in soils of culture of Tacna, Perú

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**Introduction:** Lactose (O -  $\beta$  - D - galactopyranosyl - (1 - 4) -  $\beta$  - D - glucopyranose) is the main sugar of natural origin formed by glucose and galactose present in milk and dairy products, lactase ( $\beta$ - galactosidase) accelerates the hydrolysis reaction of lactose to be ingested, but there are intolerant people and new sources of lactase from *Trichoderma* species are being sought in corn soils in Tacna, Perú.

**Methodology:** Soil samples were taken from the towns of Calana, Magollo, La Yarada 5 and 6, Tacna, Pocollay, Las Yaras, Pachía, and Los Palos. The samples were seeded on Martin agar and incubated at 25 ° C for up to 7 days. The developed colonies of *Trichoderma* were isolated and identified considering the taxonomic keys. Inocula of  $15 \times 10^6$  CFU mL<sup>-1</sup> were obtained from each isolated culture previously seeded in Potato Dextrose Agar (PDA), to be seeded in the Lactase Production Medium (LPM) fermentation medium and incubated at 28 °C with shaking for 6 days. for the production of  $\beta$ -galactosidase. When filtering and centrifuging the fermentation medium, the determination of total proteins and the enzymatic activity of the  $\beta$ -galactosidase on the ONPG (ortonitrophenyl- $\beta$ -D-alactopyranoside), lactose analog, was made in hydrolyzing it to produce galactose as products and ONP (ortonitrophenyl).

**Results:** 13 *Trichoderma* cultures were isolated, representing 20.31% of a total of 64 soil samples from the Tacna valley, 4.69% being found more frequently in the towns of Calana and Magollo. Three species were identified: *T. harzianum*, *T. aureoviride* and *T. viride*. All the *Trichoderma* investigated were producers of  $\beta$ -galactosidase being *Trichoderma* sp. TT01, isolated from Tacna, which had the highest enzymatic activity with 18,512 U mg<sup>-1</sup> protein.

**Conclusion:** It was determined that the soils of corn crops in Tacna exist *Tichoderma* sp. with lactase-producing capacity ( $\beta$ -galactosidase).

**Key Words:** Enzyme activity,  $\beta$ -galactosidase, lactose, *Trichoderma*



### III.5.3. Isolation and identification of toxin-producing fungi in macroalgae

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#### ABSTRACT

- **Introduction:** Toxin-producing fungi can become dangerous for our health when left unchecked. The objective of this research is to identify these fungi in macroalgae and identify the possible mycotoxins to further the development of ways to improve the food safety regarding their toxin-producing species, in this case macroalgae.

- **Methodology:** Samples were collected in two nearby beaches (Praia de Matosinhos, Praia da Memória) in North Portugal. The fungi are cultivated in PDA and SAB culture medium, then we proceed to extract the DNA followed by the use of PCR technique and electrophoresis to identify the fungi species after sequencing. The mycotoxin analysis is currently being done by colleagues from the University of Santiago de Compostela, Spain.

- **Results:** Several macroalgae were collected (*Laminaria sp.*, *Ulva rigida*, *Codium sp.*, *Mastocarpus stellatus*, *Chondrus crispus*, *Fucus spiralis*) and several fungi were isolated from all of them except from *Chondrus crispus*, there were no fungi growth with that macroalgae specie. Some fungi identified can potentially be pathogenic (*Alternaria alternata*, *Aspergillus fumigatus*, *Candida sp.*, *Cladosporium sp.*, *Rhodotorula mucilaginosa*).

The number of fungi isolated from the macroalgae samples from Praia de Matosinhos was far greater than the samples from Praia da Memória

-**Conclusion:** Macroalgae species, harvesting locations and water quality may influence the safety of macroalgae.

Toxin-producing fungi may be present in macroalgae, and the monitoring of fungi and mycotoxins is essential to ensure the quality of the food product.

**Key Words:** macroalgae, fungi, mycotoxins, food safety

### III.5.4. Evaluation of proteolytic activity of bacterial strains isolated from shrimp waste

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**-Introduction:** In Perú, shrimp harvest reached 50,819.62 MT in 2019 <sup>(1)</sup>; during its processing, waste are generated from exoskeleton composed mainly of proteins (40%), lipids (10%), minerals (28%) and chitin (17%) <sup>(2)</sup>. The present research aimed to evaluate the proteolytic activity of bacteria isolated from shrimp waste.

**-Methodology:** *Bacterial isolation:* Shrimp waste (RL) were cultured in seawater broth (1:5) and incubated at 25 °C for 72 h at 180 rpm. Serial dilutions were performed on seawater agar plates at the same conditions; strains were selected based in their differentiated morphological and biochemical characteristics. Plate evaluations were performed with casein (3%) and gelatin (1.5%) as substrates <sup>(3,4)</sup> to select the best proteolytic bacteria; 5 µL of the strain was inoculated and incubated at 25 °C for 120 hours. Hydrolysis of these substrates was determined by the formation of the halo of transparency around the colony. Bacteria were identified using the API20NE kit and 16S rRNA gene sequencing.

**Proteolytic activity:** The expression of proteases was induced in two different broths with casein and shrimp waste, both at 1% <sup>(5, 6)</sup> , collecting the raw extracts at 8, 24 and 48 h. Proteolytic activity of raw extracts was evaluated against azocasein. The results were quantified by Lowry method <sup>(7)</sup>.

**Zymograms:** Proteases were detected in non-denaturing polyacrylamide gels supplemented with 0.05% casein. The presence of proteases was observed as clear bands in the gel, produced by the degradation of casein <sup>(8, 9)</sup>.

**-Results:** Twelve strains were isolated from RL and belong to the genera *Shewanella*, *Bacillus*, *Acinetobacter*, *Staphylococcus*, *Brevibacterium*. The best proteolytic activity on casein were strains C12 (1.918 U/mL) and C41 (1.432 U/mL); on RL were strains C8 (1.673 U/mL) and C12 (0.455 U/mL) at 8 hours of culture for both substrates. Casein zymograms showed that strains C41 and C12 expressed proteolytic enzymes at 8 h.

**-Conclusions:** Strains of genus the *Shewanella* isolated from RL showed the highest proteolytic activity with casein and RL as substrates. The zymograms allowed to identify enzymes with proteolytic activity.

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**Key Words:** shrimp waste, microbial proteases, zymograms

### III.5.5. Effects of differing light colors on the growth and biomolecules in *Aphanocapsa holsatica*, a Cyanobacteria

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**Introduction:** Cyanobacteria is a group of organisms that can be applied in many industries as fish feed, bioremediation, biofixation food industry, among others. Light modulation is an alternative to optimization of cyanobacteria bioprocesses, as pigments, proteins and growth rate improvement; it's related on the light wavelength and microorganism metabolism. *Aphanocapsa holsatica* is a cyanobacteria usually present in blooms and it has never been studied as a source of bioproducts. The strain *A. holsatica* (3053) was isolated from Brazilian natural aquatic water reservoir and it was gently furnished for this study by the Laboratory of Phycology (UFSCar, Brazil). We aimed investigate the effects of different light colors (white as control, red, blue and green) on total proteins, chlorophyll a and total carotenoids concentrations, and dry biomass in 96 h cultures.

**Methodology:** Batch 500 mL cultures were perform in 1000 mL Erlenmeyer flasks using BG-11 as culture medium. Cultures were illuminated from the bottom up using white, red, blue and green LED with optical path of approximately 5 cm. Controlled conditions were used throughout (12:12 h light: dark cycle; 25 °C and 180 mmol fótons m<sup>-2</sup> s<sup>-1</sup> light intensity). Chlorophyll a (chl a) fluorescence, dry biomass (DB) and absorbance were daily measure. Total carotenoids were calculated according to Wellburn, (1994). Total Proteins were measured in exponentially growing cells and quantified using Slocombe *et al.* (2013) methodology.

**Results:** The short light path accomplished by illuminating the cultures from the bottom have resulted in higher growth rates as compared with the literature. Maximum growth rate of 0.40 day<sup>-1</sup> was obtained for the control. Protein concentration was highest in cells exposed to green light (69.80 µg/mL), equivalent to 47.28 % of the DB, higher than wild *Arthrospira platensis* (Spirulina) and the control. Red light was the worst for growth rate and total proteins. Green light showed to best influence carotenoids accumulation, and its concentration was 1.310 mg/g DB in comparison to 0.842 mg for the control. The ratio carotenoids: chl a in cells exposed to red light was 0.543 and in blue light 0.430.

**Conclusions:** *Aphanocapsa holsatica* (3053) showed higher biomass productivity on white light. Green light was the best color to improve both proteins and carotenoids concentrations as per unit DB. These results showed that *A. holsatica* (3053) is a good protein and carotenoids source.

**Key Words:** Cyanobacteria, Color Light, Proteins, Growth

### III.5.6. Characterization of cyanobacteria *Rhabdoderma lineare*: a physiological and biochemical approach

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#### ABSTRACT

Cyanobacteria forms of a group of abundant and diverse microorganisms. They are unicellular, photosynthetic prokaryotes, and one of the most primitive life forms on the planet. The biochemical products produced and accumulated in these organisms are of commercial interest and have applications in several areas. This study aimed to characterize the biomass of the cyanobacteria *Rhabdoderma lineare* (CCIBt-3083) concerning its growth, aspects of physiology, and biochemical composition, evaluating about its biotechnological potential and possible commercial applications. For this study, *R. lineare* was grown in a cylindrical bioreactor containing 800 mL of BG-11 medium, under air/CO<sub>2</sub> bubbling, and intensity light 250  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Microalgae cultures were kept under controlled environmental conditions and monitored daily for chlorophyll *a* fluorescence, maximum photosynthetic quantum yield, and dry biomass, during 7 days. On the last day of the exponential phase, samples were taken to determine the content of total proteins and lipids, as well as the pigments chlorophyll *a* and *b*, total carotenoids, and phycocyanin. At that time, photosynthesis analysis was performed using pulse amplitude modulated fluorescence (PAM), by determining the effective quantum yield, energy dissipation, and light curve. From these data added to chlorophyll cross section information, the theoretical CO<sub>2</sub> fixation was calculated. The results showed that *Rhabdoderma lineare* presented a growth rate of 0.47 d<sup>-1</sup>, and at the end of cultivation, the dry biomass yield was 0.54 mg/mL. Regarding the biochemical composition, total proteins content was 109.20  $\mu\text{g/mL}$  and total lipids corresponded to 6.47% of the dry biomass. The concentration of chlorophyll *a* was 1.89  $\mu\text{g/mL}$ , chlorophyll *b* 0.51  $\mu\text{g/mL}$ , and total carotenoids 0.57  $\mu\text{g/mL}$ , but phycocyanin content was below the detection limit. The photosynthetic analysis showed that the maximum yield ranged from 0.20 to 0.30, while the effective yield was  $0.18 \pm 0.004$ , the saturating irradiance ( $E_k$ ) was 247.12  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , and the theoretical CO<sub>2</sub> fixation was 0.075 g C/mg Chl *a* d<sup>-1</sup>. Given the results and comparison with literature, *Rhabdoderma lineare* is a promising species for biotechnological applications, since its biomolecules yields are equivalent to the yields of the commercial cyanobacteria, *Spirulina*.

**Key Words:** Biotechnology; Cyanobacteria; Biomolecules; Photosynthesis.

### III.5.7. Phenotypic characterization of bacteriophages isolated from wastewater against clinical strains of multiresistant *Pseudomonas aeruginosa*

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#### -Introduction:

Bacteriophages are intracellular parasites, which can infect bacterial cells. These phages are species-specific since the phages of *Escherichia coli* only could infect these bacteria and no other bacterial species. For this reason, bacteriophages are considered as a potential therapeutic tool against infections caused by multiresistant bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* y *Pseudomonas aeruginosa*.

#### -Methodology:

10 strains of MDR *P. aeruginosa* were isolated and 10 bacteriophages were obtained from wastewater for each of these strains. Subsequently, to determine the stability of the phages, they were confronted at different pH levels, high and low temperatures and ultraviolet light. And finally, its host range was determined.

#### -Results:

The *P. aeruginosa* strains collected showed resistance to the antibiotics amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, norfloxacin, piperacillin /tazobactam and tobramycin. On the other hand, the phages were stable to the different test factors and most of these phages were able to lyse 50% or more of the *P. aeruginosa* MDR strains.

#### -Conclusions:

Phages F1AO504, F1AO505 and F1AO517 showed the best characteristics since they maintained their stability against the different test factors and were able to lyse 50% or more of the tested MDR strains of *P. aeruginosa*. Therefore, these phages will be taken to the next level of research that will include molecular characterization and assays in murine models.

**Key Words:** Phage therapy; *Pseudomonas aeruginosa*, Multiresistance, Host range



**III.5.8. Antimicrobial effect in transcriptomic studies *Tarasa capitata*, *Tarasa operculata* and *Tarasa tenuis*, from the Arequipa region**

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**-Introduction:** Arequipa has a variety of plants that mostly develop in dry and desert environments, many of them have medicinal uses that have not been studied in a substantive way, this is the case of *Tarasa capitata*, *Tarasa operculata* and *Tarasa tenuis*, which according to residents of the area affirm that these have curative properties, but that in the bibliography there are only morphological descriptions, which serve to identify species. This project will determine the bactericidal effect of the extract and essence of the *Tarasa* varieties, *evaluate the minimum inhibitory concentration with extracts in Staphylococcus aureus* and *Escherichia coli* and identify the genes of the three *Tarasa* species responsible for their bactericidal compounds.

**-Methodology:** First, the biological material will be collected, the extracts and essential oils will be prepared (the hydrodistillation technique will be used) with the different parts of the plant such as the stem, the leaves and the flowers that will be made separately. Then the agar for the sowing of *S. aureus* and *E. coli* will be prepared where the extracts and essences will be placed, counting three repetitions per extract. The extracts and essences that have the greatest inhibitory effect on the different parts of the plant species will be analyzed by an Agilent 6890N Network GC System gas chromatograph (GC), coupled to an Agilent 5973 MSD mass spectrometer (MS). Then, the total RNA extraction will be carried out from the samples that show the greatest bactericidal effect using the Trizol® reagent RNA extraction kit (Life Technologies) and will be resuspended in water treated with Diethylpyrocarbonate (DEPC). After extraction, the RNA concentration obtained will be measured using spectrophotometry and the quality will be verified by 1% agarose gel electrophoresis (90V for 30 min approx.). Then, aliquots from 50uL to 60 ng/uL of the RNA samples will be prepared, stabilizing them with the RNastable kit (Biomatrica. San Diego, CA, USA) to later send them to be sequenced to the company BGI Genomic center (Shenzhen, China).

**-Results:** It is possible to find a bactericidal effect of extracts and essential oils with the expression of genes in *T. tenuis*, *T. operculata* and *T. capitata* using their stems, leaves and flowers.

**-Conclusions:** The proposed objectives will be met following the methodology procedure.

**Key Words:** Antimicrobial, essential oil, resistance, transcriptomics, *Tarasa capitata*, *Tarasa operculata*, *Tarasa tenuis*.

### III.5.9. Antimicrobial genes in 63 varieties of plants implicated in fungal, viral and bacterial diseases with attenuators of extracts, infusions and essences of plants implicated

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**-Introduction:** Microbial resistance has been a worldwide problem in recent decades, at the same time studies have been carried out with wild flora, finding some resistance to pathogens of medical importance and for agricultural production. Plant resistance to disease is experienced by the specific interaction of plant resistance genes with the corresponding avirulence genes of pathogens, genetically modified plants confer resistance against pathogens.

**-Methodology:** A search was carried out for 11 genes such as Xa21, Cf-2, RPS2, RUBISCO, intergenic spacer psbA-trnH, matK, SpaS, afp, spoNG and RPP1, where their importance, functionality and treatments that these plants can offer have been highlighted. In the form of infusions, extracts and essences, then programs such as NCBI, BLAST, MEGA, GO and VDM have been used in the latter program, most of the genes were modeled and only 3 of them did not present the necessary coverage for modeling.

**-Results:** Most of the genes show resistance to infection to pests, fungi, viruses and bacteria, attenuating diseases of animals and plants, in their entirety they activate defense signaling through associated complexes. It is also shown that transgenic plants with the AFP and RPS2 genes in *Oriza sativa* have greater resistance to bacterial diseases but in the case of Xa21 it loses functionality in *O. sativa*, resistance to microbes can not only lead to benefits in plants but also that sometimes loss of properties is seen, investigations were found where the antimicrobial effect of different species was mentioned but they were not found in the NCBI base data, in the same way the studies in which the antimicrobial effect was seen being in many cases in vegetable oils and extracts.

**-Conclusions:** It is concluded that of the revised genes all offer resistance to diseases produced by bacteria, viruses and fungi, also genetic modifications do not always confer potentiality. The aforementioned genes allow us to predict implications in the productivity of crops, mitigating pests in the field and will also allow an effective treatment of human diseases, with the use of plants, the use of toxic and harmful compounds such as insecticides and drugs that harm health will be avoided.

**Key Words:** Antimicrobial genes, bacteria, fungi, viruses, resistance, infection, health.

**III.6.1. Biostimulant property assessment of the aqueous extract from invasive alga *Caulerpa filiformis*****Sebastián Caro-Retamozo<sup>1</sup> & Edwin Ccoyllo-Terrones<sup>2</sup>**<sup>1</sup>Professional School of Genetics and Biotechnology, Faculty of Biological Sciences, UNMSM.<sup>2</sup>Laboratory of Applied Biology, Faculty of Science, UNALM, Lima – Perú.E-mail: [eccoyllo@hotmail.com](mailto:eccoyllo@hotmail.com)**ABSTRACT**

The seaweed *Caulerpa filiformis* currently represents a threat to the biodiversity of macroalgae in the Peruvian sea. However, it is also an important source of plant hormones and other bioactive compounds that can be harnessed in the form of a biostimulant; which can promote growth and boost the metabolism of crops, helping them to cope with stressful situations. This research project aims to evaluate the biostimulant potential of the aqueous algae extract on the culture of *Lactuca sativa*. For this purpose, samples of *C. filiformis*, taken out from the bay of Paracas, will be used; then it will be subjected to a drying and pulverizing process in order to obtain the dry biomass which will be used to produce an aqueous extract through the cellular disruption method by sonication. The biostimulant will be tested on *L. sativa* seeds using the "seed priming" technique. It is expected to evaluate the biostimulant activity of the aqueous extract of *C. filiformis*, obtained through an environmentally friendly method, and see if it could be scaled up to a pilot level of production.

**Key Words:** Biostimulant, *Caulerpa filiformis*, aqueous extract, *Lactuca sativa*

### III.6.2. Cyanobacterial Exopolysaccharides from production process to associated bioactivities

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#### ABSTRACT

Cyanobacteria are ubiquitous phototrophic organisms which were responsible for the Great Oxidation Event (GOE) in planet Earth 3 billion years ago. These bacteria-like organisms can grow on inorganic carbon and atmospheric nitrogen and produce a plethora of compounds. Among those, complex polysaccharides can be found as exudates known as exopolysaccharides (EPS). These excreted material can often comprise other biomolecules such as proteins, lipids and nucleic acids though polysaccharides prevail. Cyanobacterial EPS play an important role as nutrient repositories, motility and weathering processes. As a polymer, it can range from Da-MDa size and can be made of up to nine different monosaccharides and can contain sulphates, methyl, acetyl or peptides groups. The discovery of cyanobacterial EPS has been under the lights however, its structure elucidation burdens the number of known structures. Resilient cellular factories associated to functional and bioactive polymers are the hot topic on the discovery of natural polysaccharides.

In order to develop a sustainable bioprocess, EPS and biomass yields are crucial in the selection of the strain but also ability to handle fluctuating abiotic factors. The variation of culture conditions has helped in understanding which factors could be determinant for the EPS production while the downstream processing can vary according to the location of the EPS. Notwithstanding, biological activity has been extensively reported but not often combined with the sustainability of the production process. Rare sugars and substituent groups are reported to possess antioxidant, anti-cancer, immune-stimulatory and anti-inflammatory properties. An overview of the cyanobacterial EPS from the production process to the bioactive profile will be here explored.

**Key Words:** Cyanobacteria, Exopolysaccharides, Biopolymers, Bioactive profile

### III.6.3. Microalgae and Cyanobacteria Biostimulant Effects in the Germination of Seeds

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#### ABSTRACT

The increasing world population and the damage caused by conventional agricultural practices, using pesticides and chemical fertilizers in excess, cause enormous impacts in environment and human health. This is the main propulsor for the research of alternatives that lead to sustainable and healthy agro-practices. Microalgae and cyanobacteria have been gaining ground to help combat this issue and have a great potential to become the future of plant stimulants, fertilizers and bioprotectors. Apart from the nitrogen and phosphorus they may provide, they produce a huge variety of secondary metabolites that may increase plants growth and have protective properties against disease and stress. Their extracellular polymers may also help to conditionate soils, in special to fight drought and climate changes. In this review we will refer to the bioassays that can be used to assess these aspects. A crucial step in plants life cycle is the germination of seeds as it can determine the entire future success of a crop, making the stimulation of the germination of high importance. Microalgae and cyanobacteria are a reliable source of biomass, economically viable and will provide sustainable alternatives to chemical stimulation.

**Key Words:** microalgae, cyanobacteria, biostimulant, germination.



### III.6.4. Discovery of novel biological active metabolites from cyanobacteria and actinobacteria: Cytotoxic, anti-obesity and appetite reduction effects

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#### ABSTRACT

Obesity is a complex metabolic disorder related to co-morbidities, such as hepatic steatosis, cardiovascular diseases, diabetes mellitus, chronic inflammation, and some types of cancer. With the increase in the prevalence of obesity and the reduced medication available to reverse the disease, research in other alternative sources is crucial to urgently find new-generation and effective pharmaceuticals with restricted secondary side effects. Microorganisms' secondary metabolism is a valuable source of novel natural products (NP) with interesting pharmacological applications. This work aims to identify novel bioactive compounds, such as pharmaceuticals and/or nutraceuticals from cyanobacteria from LEGE-CC collection, and actinobacteria isolated from a species of Marine Sponge, *Hymeniacidon perlevis*, for metabolic diseases, with a focus on finding novel molecules for obesity and related diseases, appetite and cancer. To date, were performed anti-obesity assays by Nile Red Fat metabolism assay for the screening of seventy-nine extracts of marine sponge-associated actinobacteria. One extract induced the lipids accumulation in zebrafish larvae, supposedly via PPAR $\gamma$ , and for this extract, the evaluation of anti-diabetes activity is ongoing. Appetite reduction assays using fluorescent stained *Paramecia bursaria* were also executed, testing fifty-seven cyanobacterial fractions, and three fractions demonstrated promising appetite reduction activity, while another induced the appetite. The screening of actinobacterial crude extracts is ongoing for both appetite assays, and with liposomes for cyanobacterial fractions. Cytotoxic activity of actinobacterial crude extracts was evaluated in two human cancer cell lines (human liver cancer HepG2 and human colorectal cancer HCT-116) and in non-carcinogenic endothelial cell line (hCMEC/D3) using the MTT method, being that forty-three extracts exhibited significant effects on the viability of at least one tested cancer cell line and only nineteen extracts revealed activity against hCMEC/D3. In the future will be performed dereplication by metabolomics (LC-MS/MS), as well bioactivity-guided feature based molecular networking using GNPS, to highlight putative associated metabolites for the bioactivities.

**Key Words:** metabolic diseases, zebrafish larvae, natural products, anti-obesity, appetite

### III.6.5. Growth and biochemical characterization of four Chlorophyta microalgae

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#### ABSTRACT

Algal biotechnology has been attracting more attention in the past years because of their industrial applicability in reason to the high-value compounds they produce and accumulate. However, microalgae remain as a barely explored group of microorganisms. Thus, prospecting new species can help in the search for promising organisms. Aiming to contribute to the knowledge of microalgae biochemical characteristics, this work investigated some Brazilian microalgae strains (*Ankistrodesmus arcuatus*, *Elakatothrix* sp., *Radiococcus* sp., *Sorastrum bengalicum*), as far as we know, not studied before. The best light intensity for growth kinetics, biochemical analyses as total biomass, lipids, proteins, carotenoids, and chlorophylls were determined. Batch cultures were performed under laboratory-controlled conditions and samples for the analyses were obtained in exponentially growing cells, what coincided with the third or fourth day of the cultivation, depending on the microalgae. Light saturation intensity was lowest for *A. arcuatus* ( $170 \mu\text{molm}^{-2}\text{s}^{-1}$ ), within 230 - 270 for the other algae. The lowest total carotenoids content was present in *A. arcuatus* ( $0.016 \mu\text{g/mL}$ ), while the others had it in the range  $0.048\text{-}0.080 \mu\text{g/mL}$ . These results indicate that light resistance is related to the carotenoid content. The growth rates obtained in the exponential phase were 0,57, 0,81, 0,98 and 0,79, for *Ankistrodesmus arcuatus*, *Elakatothrix* sp., *Radiococcus* sp., *Sorastrum bengalicum*, respectively. Dry weight in the 4th culture day ranged from  $0.013 \text{ mg/mL}$  (*S. bengalicum*) to  $0.019 \text{ mg/mL}$  (*A. arcuatus*), and *Radiococcus* sp and *Elakatothrix* sp produced  $0.015$  and  $0,016 \text{ g/mL}$ , respectively. Regarding the percentage of lipids and proteins by the dry weight, the strain *A. arcuatus* had the highest lipid content (24.6%) and only 17.64% of proteins. *S. bengalicum* had the second highest percentage of lipids (18.3 %), and a protein concentration of 23.31%. *Elakatothrix* showed 16.26 % of lipids and for the percentage of proteins (25.19 %), and, lastly, *Radiococcus* showed 28.39 % of proteins and 14,40 % lipids. These results are a contribution to the prospection of Brazilian diversity, and microalgae in general, hoping to provide information that can be useful to the microalgae biotechnological sector.

**Key Words:** microalgae, carotenoids, biomolecules, growth rates.

### III.7.1. The potential of marine cyanobacteria as a source of lipid-reducing metabolites

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#### ABSTRACT

Obesity is a complex metabolic disease that became a major public health concern worldwide. In the last decades, research has focused on natural product discovery as a novel therapeutic strategy to overcome this epidemic. Recent reports have shown that the marine environment is an underexploited rich source of prolific natural compounds, and cyanobacteria are regarded as a high valued source of bioactive secondary metabolites.

A bioassay-guided approach was used to explore the potential of secondary metabolites with lipid-reducing activity from marine cyanobacteria from LEGE-CC (Blue Biotechnology and Ecotoxicology Culture Collection) hosted by CIIMAR.

The cyanobacteria *Nodosilinea* sp. LEGE 06001 yielded a new bioactive chlorophyll derivative, 132-hydroxy-pheofarnesin a. The structure elucidation was established based on NMR spectroscopy and mass spectrometry. This compound showed significant neutral lipid-reducing activity in the zebrafish Nile red fat metabolism assay after 48 h of exposure with a half maximal effective concentration (EC<sub>50</sub>) of 15.5 ± 1.3 µM.

Furthermore, bioactivity screening for anti-obesity activity within the European ERA-NET MB CYANOBIOSIS project framework indicated another promising strain with great lipid reducing activity. So, additional bioassay-guided isolation efforts are performed on the strain *Cyanobium* sp. LEGE 06097. Several (sub)fractionation steps were employed to fully purify and identify the active metabolite(s). In addition, an untargeted metabolic approach was used to boost the identification of the responsible bioactive molecule(s).

In summary, a novel and other(s) unidentified metabolite(s) were discovered from marine cyanobacteria with relevant lipid-reducing activities, which in the future may be developed into nutraceuticals.

**Key Words:** Cyanobacteria; Obesity; Zebrafish assays; Nutraceuticals.

### III.8.1. Optimization of the production of silver nanoparticles mediated by aqueous extracts of açai (*Euterpe oleracea*)

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**Introduction:** The production of metallic nanoparticles (MNs) by different routes apart from the traditional ones has been emerging in the field of nanotechnology, especially those using concepts of Green Chemistry to minimize potential damages to the environment. Thus, approaches using phytochemicals found in plant extracts are presented as a possibility to obtain MNs. The Amazon region has an ecosystem rich in biodiversity, and its fruits, such as açai (*Euterpe oleracea*) can exhibit antioxidant properties associated with their chemical composition. In açai there are phytochemical species that might act as reducing agents and stabilizers of NMs, and therefore this study aims to investigate the optimization of the production of silver nanoparticles (AgNPs) mediated by aqueous extracts of açai under different reaction conditions.

**Methodology:** The methodology was divided into 3 steps: obtaining and characterizing aqueous açai extracts by UV-Vis spectroscopy; the production of AgNPs under different concentrations of açai extract and monitoring the progress of reaction by UV-Vis spectroscopy; the production of AgNPs in acidic (pH 6), neutral (7) and alkaline (8) media, monitoring the progress of the reaction by UV-Vis spectroscopy.

**Results:** Two intense bands in the UV-Vis spectrum of açai extract were identified in the range of 200-300 nm which were attributed to the phenolic compounds found in these aqueous extracts. It was possible to produce AgNPs under more dilute extract conditions and at pH 6, evidenced by the change in the color of the reaction medium characteristic of nanoparticle formation, as well as the visualization of the band associated with surface plasmon resonance (SPR) located between 350-450 nm. The SPR band was not observed in reaction media containing higher concentrations of açai extract. For the reaction vessels at higher pH values and more dilute extract conditions, a higher reaction rate was observed. However, it was also noticed the presence of reduced silver precipitates, which may be related to the low kinetics of interaction between the biomolecules of the extract and the crystals of AgNPs formed.

**Conclusion:** The production of AgNPs mediated by açai extracts occurred with greater efficiency under more diluted extract conditions and lower pH values of the reaction media due to the compositional characteristics of this fruit.

**Keywords:** Nanotechnology; alternative routes; green synthesis; plant extracts.

### III.8.2. Production of silver nanoparticles mediated by aqueous extracts of tucumã (*Astrocaryum aculeatum*)

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**-Introduction:** In a scenario of greater concern about the impact of human action on the environment, nanotechnology can take advantage of the use of environmentally less harmful species at different stages in the production of materials with new and/or enhanced properties in comparison to those with micro/macroscopic dimensions. Among the various metallic nanoparticles, silver ones stand out mainly owing to their microbiocidal properties. Considering the different methods of preparing silver nanoparticles (AgNPs), the biological route becomes an interesting alternative due to its low environmental impact and the use of renewable raw materials in these processes. Plant extracts have shown promising results in the production of AgNPs, in addition, there are a large number of plant species in the Amazon region whose extracts (stem, fruit, seed and leaf) can be used as bioreducing agents in alternative synthetic routes. This study investigated the formation of silver nanoparticles by aqueous extracts of tucumã pulp (*Astrocaryum aculeatum*) under different conditions, evaluating the concentration and pH parameters of the reaction media.

**-Methodology:** The methodology was divided into 3 steps: obtaining and characterization of aqueous extracts of tucumã pulp by UV-Vis electronic spectroscopy; synthesis of AgNPs under different concentration conditions of tucumã extract, and monitoring of reaction progress by UV-Vis spectroscopy; obtaining AgNPs in acid (pH 5), neutral (pH 7) and alkaline (pH 9) media, monitoring the progress of the reaction by UV-Vis spectroscopy.

**-Results:** Low concentrations of plant extract in reaction media were not enough to reduce all silver cations present. On the other hand, an increase in the concentration of phytochemicals in the medium can generate a competition between the bioreduction process and the complexation of metallic cations. Reaction media with higher pHs generated faster, more defined, more intense and shorter wavelength signals in UV-Vis spectroscopy, indicating the formation of more uniform and smaller AgNPs. These results indicate that the formation of AgNPs is influenced by both experimental parameters.

**-Conclusions:** The optimized production of AgNPs mediated by tucumã extracts occurred under moderate concentration of pulp extract and in basic media due to the compositional characteristics of this fruit.

**Keywords:** Nanotechnology; microbiocidal activity; green synthesis; plant extracts.



### III.8.3. Removal of Methylene Blue from Synthetic Wastewater Using Ingá Bark as a Low Cost Biosorbent

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**-Introduction:** Water is essential for all forms of life. However, with the development of the industry, many pollutants (such as synthetic dyes used in the textile, paper and printing industries) are directly or indirectly released into the water, causing serious pollution. An alternative for removing these contaminants is through adsorption. At that point, natural adsorbents have been heavily studied. Ingá (IG) is a very popular fruit in the Amazon region, and is not exclusive to Brazil. The *Inga edulis* species belongs to the legume family, it has been widely used in orchard planting and in agricultural ecological management, it is a good nitrogen fixative and very suitable for shade.

**-Methodology:** The isothermal experiments were carried out in triplicate (standard deviation  $\pm 0.1$ ) with 25 mL of methylene blue (MB) solution at  $4.79 \times 10^{-4}$  to 23.95 g L<sup>-1</sup> concentrations, 200 mg of the shells of inga and 6.4 pH at 25 °C to 55 °C in contact for 60 minutes, and analyzed by UV-Vis. For desorption studies, 25 mL of MB aqueous solution (24.58 mg L<sup>-1</sup>) were used in contact with 200 mg of Ingá for 60 min under stirring, and after this was dried at 90 °C for 12 h and placed in contact with 25 mL of ethanol at pH 6.4 under stirring for 10 min.

**-Results:** The equilibrium data for this system at 25 °C were explained by Langmuir and Freundlich isotherm. For the Langmuir the RL value was 0.72, indicates the adsorption has been favorable. The Freundlich constants Kf and nf were 2.1 and 40.98 respectively, indicating that adsorption is the physical process and the homogeneous behavior of the surface. The data show that at 298 K, the capacity of adsorption of the MB onto IG increased from 82,77 to 99,23%, in the 1st and 2nd cycle, respectively at 2 min of contact. For the desorption property, it can be observed a similar situation, i.e., the desorption percentage increased after those 2 cycles (12,45% and 25,33%).

**-Conclusion:** The results obtained show that IG can be applied as a low-cost adsorbent to remove MB from effluents. As a waste product, it provides a possible solution for solid waste management. The MB adsorption process indicated a good fit to the Freundlich isothermal model at 25°C.

**Key Words:** Bioadsorption; Dyes; Ingá; Isotherms.

### III.8.4. Obtaining micro and nanocellulose from lignocellulosic biomass from Ingá-Cipó waste (*Inga edulis* Mart.) by chemical treatment

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**Introduction:** The relentless search for new sustainable materials causes a gradual increase in studies aimed at the use of renewable raw materials, seeking to generate environmentally friendly products, maintaining their quality and performance, an example is the extraction of nanocellulose from plant material and its incorporation into matrices for the manufacture of composites. Fruits generate residues that are great sources of raw material such as peel and bagasse, the fruit of the ingá-vine (*Inga edulis* Mart.), is made up of rind, pulp and seeds, with the rind being the major component of the fruit, the extraction of nanocellulose from this type of material is already widely explored and daily gains more industrial applications. Therefore, the work aimed to extract micro and nanocellulose from the residual ingá-vine bark.

**Methodology:** The husks were washed and pulped in running water and dried in an oven at a temperature of 60° C for 24 hours, then the material was crushed in a knife mill and sieved in a 60 mesh granulometric sieve. Subsequently, the material was subjected to a sequential extraction initially with distilled water followed by a treatment with 2 M sodium hydroxide (NaOH), After this initial treatment, the material was washed with distilled water to a pH close to 7, to carry out acid hydrolysis using sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) at 64% to obtain micro and nanocellulose, sequentially 0.5 L of distilled water was added to the hydrolyzed material, which was filtered and centrifuged. The bottom body was collected in a Becker where bleaching was performed using 2.5% sodium hypochlorite.

**Results:** Through the experiments carried out, a very fine material was obtained, which is believed to be micro and nanocellulose, this material will be submitted to Infrared analysis with Fourier Transform, DRX, Scanning and Transmission Electron Microscopy.

**Conclusion:** Given the above, it is concluded that the applied methodology proved to be effective for the realization of the present work and with the future analyzes that will be carried out, it will be possible to say if the extracted material is really micro and nanocellulose.

**Key Words:** *Inga edulis*, extraction, nanotechnology, biotechnology

**III.8.5. Antioxidant activity of the fern *Lophosoria quadripinnata* (J.F.Gmel)  
C.Chr.: a potencial resource for phytotherapy**

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**-Introduction:** *Lophosoria quadripinnata* is a fern species of the family Dicksoniaceae, widely distributed in central and southern America. In Chile, it is found in the southern zone with abundant populations in native forests, scrublands and cultivated in urban gardens. It is part of the medicinal flora used by native peoples for its homeostatic activity. The objective of this work was to determine the content of phenolic compounds and the antioxidant activity of the aqueous extract of *L. quadripinnata*.

**-Methodology:** Leaves of *L. quadripinnata* were collected in the rural area of the city of Valdivia, Chile; the material was crushed, weighed 2.5 grams, and added to 250 mL of distilled water for extraction; the solution was sonicated, filtered, and the extract obtained was lyophilized. The following tests were performed with the resulting solid: ferric reducing/antioxidant power (FRAP), expressed in micromoles of Trolox equivalents per gram of extract; 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) retention, expressed as the mean maximum inhibitory concentration (IC<sub>50</sub>); the free-radical trapping capacity (ORAC), expressed in micromoles of Trolox equivalents per gram of extract; determination of total phenols (FeT), expressed in milligram equivalents of gallic acid per gram of extract; and determination of total flavonoids (FlaT), expressed in milligram equivalents of quercetin per gram of extract.

**-Results:** In the FRAP assay, 2110.051 µmolET/g were obtained; for DPPH, an IC<sub>50</sub> value of 78.137 µg/mL was obtained; in ORAC, a value of 2631.432 µmolET/g was obtained; for FeT, 49.029 mgEAG/g was calculated; and for FlaT, 91.307 mgEQ/g was obtained. These values are similar to those reported for other fern species of the genus *Blechnum* and *Gleichenia* that are of medical and food important.

**-Conclusions:** This fern represents a promising resource in phytotherapy due to optimal levels of phenolic compounds that support its antioxidant activity.

**Key Words:** Fern, *Lophosoria*, antioxidant, phytotherapy.

### III.8.6. Transcriptomic analysis on the influence of the microalgae *Chlorella sp.* during the germination of *Arabidopsis thaliana* in water samples from the Tambo River contaminated with As and B

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**-Introduction:** According to the latest reports in the Arequipa Region, they mention that the waters of the Tambo River are contaminated with arsenic (As) and boron (B). Both As and B persist in ecosystems due to their non-biodegradable characteristics. The ecotoxicology of As causes decreased production of red and white blood cells, nerve damage, stomachaches, and can even damage DNA. Among the effects of B, in humans they affect the digestive, excretory and nervous systems that eventually lead to death.

RNA-seq is a current transcriptomic tool that is based on cDNA sequencing based on the development of Next Generation Sequencing (NGS). One of the fundamental steps in transcriptomics is the final obtaining of a good quality RNA that represents the final condition of the study organism.

The microalgae *Chlorella sp.* for being a cosmopolitan organism and *Arabidopsis thaliana* because its genome is 100% molecularly known.

**-Objective:** To evaluate the number of genes transcribed in the influence of the microalgae *Chlorella sp.* during the germination of *Arabidopsis thaliana* in water samples from the Tambo river contaminated with As and B.

**-Methodology:** Evaluation of As and B concentrations in water samples from the Tambo River (study area and obtaining samples of waters contaminated with As and B). Later the evaluation of the growth of *Chlorella sp.* as substrate and germination of *A. thaliana* during the effect of the extract of the Tambo river water samples contaminated with As and B. Determination of photosynthetic efficiency in the influence of the microalgae *Chlorella sp.* during the germination of *A. thaliana* in water samples from the Tambo River (quantification of carotenoids and chlorophylls "a and b"). Development of a local database of genes (obtaining readings by BGI Genomic-China) related to the key metabolic and photosynthetic processes of *Chlorella sp.* and *A. thaliana* exposed to waters of the Tambo River during plant germination.

**-Results:** A local database of genes related to the key metabolic and photosynthetic processes of *Chlorella sp.* and *A. thaliana* exposed to waters of the Tambo river contaminated with As and B.

**-Conclusions:** The proposed objectives will be met as the indicated methodology (experimental plan) is fulfilled.

**-Keywords:** Transcriptomic, Tambo river, *Chlorella*, *Arabidopsis thaliana*.



### III.8.7. Determination of bioaccumulation of heavy metals Arsenic (As) and Boron (B) in *Arabidopsis thaliana* and *Chlorella sp.* of surface waters of the Rio Tambo, Islay. 2021

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**-Introduction:** The main economic activities carried out in the Tambo Valley are agriculture and livestock, whose only source of supply is the water from the Tambo River, located in the province of Islay. In addition, the presence of heavy metals, specifically Arsenic (As) and Boron (B) within the waters of the Tambo River becomes very probable since its watersheds, the Coralaque and Omate-Carumas Rivers, present high As contents due to flows of volcanic origin, which causes progressive contamination until reaching the river. Inorganic As and B in surface waters can cause chronic intoxication in people, since river water is used for human consumption, irrigating crop fields, etc. For this reason, consider proposing clean technologies that can deal with this problem and solve it in a viable and sustainable way. Therefore, the present work seeks to determine the bioaccumulation capacity of As and B of two microorganisms of different classes, which are: *Arabidopsis thaliana* (Plant) and *Chlorella sp.* (Microalgae), in the surface waters of the Rio Tambo.

**-Methodology:** Determination of the concentrations of As and B present in the surface waters of the Rio Tambo, which will be used by the total reflection X-ray fluorescence spectrophotometer (TXRF). Cultivation of *A. thaliana* seeds according to (Sánchez-Bermejo, et al., 2014) and cultivation of pure colonies of microalgae *Chlorella sp.* Two sampling points, one at the beginning or entrance to the Tambo valley (P1) and another near the river mouth (P2). The percentage of bioaccumulation will be evaluated by means of a DCR identifying the initial and final concentration of As (III) and Boron (B), as a response variable, both for both species, and samples of the final concentration of the metals will be taken at different times (24, 48, 96 and 168 hours). This design will be used for both the P1 and P2 samples separately (tripled). For the analysis and comparison of the results between the bioaccumulation capacity between both species, ANOVA and Tukey will be used, with the use of statistical packages Minitab 19 and R

**-Results:** *A. thaliana* and *Chlorella sp.* have a greater capacity for Bioaccumulation of As and B, reducing the concentration of these compounds in surface waters of the Rio Tambo.

**-Conclusions:** The proposed objectives will be met as the indicated methodology (experimental plan) is fulfilled.

**-Key Words :** Bioaccumulation, Río Tambo, *Chlorella*, *Arabidopsis thaliana*.



### III.8.8. Influence of the extract of *Chlorella sp.* on the germination and growth of *Arabidopsis thaliana* under stress by arsenic and boron cultivated with the waters of the Tambo river

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**-Introduction:** According to the latest research, it is mentioned that the Tambo river basin has levels of arsenic (As) and boron (B) above the Environmental Quality Standards, a product of hot springs of the Vagabundo river, the volcanic material, the mining and agricultural activities. As is a highly toxic metalloid that seriously affects human health and the growth and performance of cultivated plants. B is an element that is widely disseminated in the environment and its phytotoxic, teratogenic and reproductive effects on aquatic and agricultural ecosystems are of interest. *Arabidopsis thaliana* represents an ideal organism for this type of study, in addition to the deep knowledge of its physiology.

*Chlorella sp.* will be chosen as the study material due to its wide geographical distribution, rapid spread, sensitivity to toxins and whose aqueous extracts could contain different metabolites and phytohormones that stimulate the germination and growth of *A. thaliana* under stress conditions by As and B. Later to be used in the production of different crops as an economic alternative which allows achieving sustainable agriculture.

**-Objective:** Evaluate the effect of the extract of *Chlorella sp.* on germination and growth *Arabidopsis thaliana* under conditions of Arsenic and Boron stress.

**-Methodology:** *Chlorella* samples collected from the Arequipa golf club will be used, axenic cultures will be obtained and they will be cultivated at different concentrations of As and B, to later obtain the extracts by mechanical lysis and sonication. *A. thaliana* seeds will be cultivated in MS medium under stress conditions with As, B and *Chlorella* extracts, finally, 14 days after treatment, the germination percentage and plant growth parameters will be measured.

**-Results:** It is expected to verify the biostimulant effect of *Chlorella* extract on the germination and growth of *A. thaliana*.

**-Conclusions:** The objectives will be met as the indicated methodology is met (experimental plan).

**-Key Words:** Arsenic, Boron, *Chlorella sp.*, *Arabidopsis thaliana*

### III.8.9. Determination of photosynthetic efficiency in the influence of the microalgae *Chlorella sp.* during the germination of *Arabidopsis thaliana* in water samples from the Tambo River contaminated with As and B

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**-Introduction:** According to the latest reports in the Arequipa Region, they mention that the waters of the Tambo River are contaminated with arsenic and boron from the mining sector, agriculture sector and health sector. Microalgae like *Chlorella sp.* have shown a high metal-binding capacity, in this way, heavy metals can be sequestered by the cell walls of *Chlorella sp.* and therefore its biomass is considered highly effective and reliable in removing heavy metals from aqueous solutions. *Arabidopsis thaliana* is a model organism of the most important and studied in research on molecular biology, genetics and plant physiology. It is very important to know the photosynthetic efficiency (light conditions), the light regime determines the quantity and quality of light energy available for photosynthetic organisms to conduct their metabolic activities, fulfilling a fundamental role on the production of pigment microalgae (carotenoids, chlorophylls "a" and "b"). In this sense, although there is an important informational collection in the Arequipa Region and focusing on the issue of determining photosynthetic efficiency, the work carried out can be considered preliminary, too separate or have not been studied on a large scale. For this reason, it is necessary to establish or formulate an adequate methodology for the determination of photosynthetic efficiency in the influence of the microalgae *Chlorella sp.* during the germination of *A. thaliana* in water samples from the Tambo River contaminated with As and B. All of the above adds to the reason that both species should be more valued in our Arequipa Region, since they have a high potential for good work research.

**-Objective:** To determine the photosynthetic efficiency in the influence of the microalgae *Chlorella sp.* during the germination of *A. thaliana* in water samples from the Tambo River contaminated with As and B.

**-Methodology:** Experimental study (obtaining *A. thaliana* seedlings, *Chlorella sp.* Concentration, initial As and B concentration ppm). Determination of the photosynthetic activity of *Chlorella sp.*

*Chlorella sp.* carotenoid quantification (to be determined using a formula). Quantification of chlorophylls "a" and "b" of *Chlorella sp.* (to be determined using a formula). For the data collection strategy, the equipment will be used to measure photosynthetic efficiency and spectrophotometric methods will also be applied.

**-Results:** Establish or formulate an adequate methodology for the determination of photosynthetic efficiency in the influence of the microalgae *Chlorella sp.* during the germination of *Arabidopsis thaliana* in water samples from the Tambo River contaminated with As and B.

**-Conclusions:** The proposed objectives will be met as the indicated methodology (experimental plan) is fulfilled.

**-Key Words:** Efficiency, Tambo River, *Chlorella*, *Arabidopsis thaliana*.

### **III.8.10. Four micrografting modalities and three auxins (NAA, IAA, IBA) in the in vitro management plants of lemon tree (*Citrus aurantifolia* Christm.) and orange (*Citrus sinensis* L.)**

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#### **ABSTRACT**

The research was conducted in laboratory of plant tissue culture in vitro founded "La Católica" of the UCSM in order to assess the response of four forms of micrografting (slit, excision, inverted T and T normal) with three auxins NAA (Naphthaleneacetic acid) 10 ppm, IAA (Indole acetic acid) 25 ppm, IBA (Indole butyric acid) 50 ppm and four dive times of the apices meristematic graft (5, 15.30 and 60 minutes) in the solutions of auxins. After 30 days will be proceeded to its evaluation found that the micrograft slit introduced a apprehension of 60% for lemon and 66% for Naranjo. In the effect of auxins it was observed that the IBA with a dive time of 15 minutes achieving 92% for lemon and 96% for Naranjo. Acclimation in plants treated with IBA and 15 minutes of immersion reached 30 days 53.8 % and 57.14 % of survival for lemon and orange. The conclusions were: The slit micrograft modality was the one that presented the highest percentage of seizure with 60% for subtle lemon on rough lemon and 66% for sweet orange on Cleopatra mandarin. On the use of IBA, IAA and NAA in four immersion times of the graft apices (5, 15, 30 and 60 minutes) it was found that: With IBA (50 ppm) and 15 minutes the highest percentages of seizure were obtained, 92% for subtle lemon on rough lemon and 96% for sweet orange on Cleopatra mandarin, followed by treatment with IAA (25 ppm) with statistically similar percentages ( $p > 0.05$ ) for the different times, both for lemon and for from the acclimatization in the greenhouse: The best response was obtained with the seedlings treated with IBA (25 ppm) and 15 minutes of immersion, achieving 53.84% in lemon tree and 57.14% of acclimatized seedlings in orange tree 30 days after evaluation. To complement the work, a molecular test could be carried out that allows us biological indexing, this is widely used in certification programs and is considered a sensitive and reliable method for the detection of new or unusual strains of the virus.

**Key Words:** micrograft, auxins, dip, seizure.

### III.8.11. Evaluation of antioxidant activity and polyphenol content of 7 species of microalgae

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#### -Introduction:

Microalgae are potential source of bioactive compounds and their consumption can have several benefits. Part of these benefits are due to its antioxidant activity and the presence of phenolic compounds. However, few genera have their bioactivity explored and the search for new promising species can bring potential new products. The aim of this study was to evaluate the antioxidant potential of 7 species of microalgae.

#### -Methodology:

The species *Chlorolobion braunii*, *Hariotina reticulata*, *Monoraphidium arcuatum*, *Monoraphidium indicum*, *Monoraphidium griffithii*, *Monoraphidium pseudobraunii* and *Selenastrum gracile* were chosen for this study. Biomass used for extracts was obtained from batch cultures in BG11 medium, after 8 days in the stationary phase. Extracts were obtained with 50 mg/mL of lyophilized biomass in sequential mode in the following solvent order: chloroform: methanol (2:1) (CM), methanol (M) and water at 80 °C (A). The extraction was done in triplicate with 24 hours of contact solvent/biomass, except of the last extraction, which had 30 minutes of contact only. The extraction index, the antioxidant activity by the DPPH method and polyphenol content were measured.

#### -Results:

Among the evaluated solvents, the one with the highest antioxidant activity values was chloroform: methanol with an average inhibition index of  $35.49 \pm 8.13\%$ , followed by methanol ( $2.34 \pm 1.18\%$ ) and water ( $1.47 \pm 0.16\%$ ). These values are proportional to extraction indexes and polyphenols. Evaluating the algae individually, *M. pseudobraunii* presented the highest antioxidant activity value ( $49.69 \pm 3.61\%$ ) in the CM extract, followed by *M. arcuatum* ( $41.60 \pm 3.33$ ), *M. indicum* ( $36.52 \pm 2.22$ ) and *M. griffithii* ( $35.34 \pm 1.18$ ). Similar behavior was not observed for the more polar extracts, indicating a possible correlation between genus and the presence of less polar antioxidants compounds. For methanol extracts, the highest inhibition index was observed for *S. gracile* ( $28.14 \pm 4.97$ ). These extracts showed a linear correlation between the increase in antioxidant activity and the polyphenol content  $R^2 = 0.81$ . Most of the aqueous extracts did not show activity and the highest value was obtained for *C. braunii* ( $6.62 \pm 1.30$ ), which indicates that most of the compounds with antioxidant activity were extracted with previous solvents.

#### -Conclusions:

All microalgae showed some antioxidant potential, but the highlight goes for the genus *Monoraphidium sp.*. It can be concluded that the combination of the CM and M sequence was efficient in extraction of most antioxidant compounds and that the M extracts showed a direct relationship between antioxidant activity and polyphenol content.

**Key Words:** DPPH; Bioactive Compounds; Chlorophyta; Extraction.

**III.9.1. In vitro anticancerigenous effect of different extracts of microalga *Dunaliella salina* (Teodoresco) on colorectal adenocarcinoma cell line (SW48)**

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**ABSTRACT**

Cancer and its subtypes constitute a relevant and vitally important health problem in the world and in the Peruvian population, being colorectal cancer (CRC) one of the types of cancer that seriously affects the human health. Early stage CRC is curable; however, when it reaches a stage that involves metastasis (mCRC) it can represent a severe condition and its treatment is subject to the size, location and extent of the cancer. One of the main cancer-inducing phenomena is the malfunctioning of the cell cycle, alterations in DNA methylation, variations in the expression of certain suppressor genes and oncogenes, histone modification and non-coding ribonucleic acids (RNA). In recent years, a trend has emerged in the study of bioactive metabolites from marine sources, particularly microalgae. *Dunaliella salina*, a microalgae found in the salt flats of Chilca Perú, contains chemical compounds or secondary metabolites, which have been evaluated in early stages of cancer showing positive effects. However, its properties still need to be studied in metastatic cancer stages such as in the case of colorectal adenocarcinoma, Dukes type C, grade IV. In this context, it is necessary to study this disease from a perspective based on biomolecules that allow its inhibition. The aim of this research is to determine the in vitro anticarcinogenic effect of different extracts of microalgae *D. salina* (Teodoresco) on the colorectal adenocarcinoma cell line (sw48). Materials and methods: The microalgae culture will be developed in Johnson's medium, under conditions of salinity stress and nitrogen reduction in a bioreactor. Five types of *Dunaliella salina* biomass extracts will be obtained using solvents of different polarity (water, ethanol, ethyl acetate, n-heptane and acetone). Cytotoxicity assessment on the cell line will be carried out by tetrazolium salts (MTT), gene expression analysis of tumour suppressor genes (APC, ASSF1A and MCC) as well as DNA methylation (MLH1, MGMT and MSH2 genes) will be carried out by real-time PCR (RT-QPCR). The determination of the chemical profile of the active extract of *D. salina* will be performed by HPLC with mass spectrometry detector.

**Key Words:** microalgae, *Dunaliella salina*, colorectal adenocarcinoma (SW48), extracts, tumour suppressor genes, DNA methylation, real-time PCR.



### III.9.2. Evaluation of the cytotoxicity of chlorophormic extract and chromatographic fractions of *Dracontium spruceanum* on gastric cancer cells (AGS cell line)

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- **Introduction:** *Dracontium spruceanum* is a plant distributed mainly in countries that share the Amazon rainforest in South America. This has been used for various purposes such as treating snake bites or hernias. The effect of different extracts of this plant has recently been evaluated; however, the cytotoxic activity of fractions obtained by column chromatography in gastric cancer cells is not yet known. Therefore, the objective of the present study was to discriminate which of the chromatographic fractions have a cytotoxic effect on the AGS cell line.

- **Methodology:** The AGS cell line was cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum and 1% antibiotic and incubated with 100 µg/mL of the chlorophormic extract of *Dracontium spruceanum*. The chlorophormic extract was fractionated by column chromatography and the cytotoxicity of 11 chromatographic fractions was evaluated. Cell viability was quantified by the MTT assay at 24 h and 48 h of exposure.

- **Results:** The cell viability of AGS, gastric cancer cells, exposed to the chlorophormic extract of *Dracontium spruceanum* was reduced to  $31.9 \pm 4.9\%$  at 24h and to  $22.5 \pm 2.8\%$  at 48h, compared to the control group. Chromatographic fractions F50 and F52 had a significant cytotoxic effect on AGS cell viability, at 24h viability was reduced to  $12.6 \pm 1.6\%$  and  $17.4 \pm 2.1\%$  respectively; and at 48h it was reduced to  $14.3 \pm 5.2\%$  and  $18.1 \pm 6.8\%$  respectively, both compared to the control group.

- **Conclusions:** The fractions obtained by column chromatography with the greatest cytotoxic effect were F50 and F52. These results show that there are compounds in the chlorophormic extract of *Dracontium spruceanum* that require further study and that when separated by column chromatography they would have increased biological activity

**Key Words:** *Dracontium spruceanum*, chromatographic fractions, cytotoxicity, MTT, gastric cancer.

### III.9.3. Iron supplementation and its effect on the composition of the gut microbiome in 5-month-old infants

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**Introduction:** Anemia is the most widespread public health problem in the world. More than 30% of the world's population has some degree of anemia, the most common cause being an iron deficiency. During the early stages of life, anemia can have negative impacts on a baby's growth and neurological development. Therefore, it is currently recommended to add iron supplements to the diet of all babies who were 4 months old, to prevent anemia. However, this supplementation was observed to be excessive and had several adverse effects, including changes in the gut microbiome, which influences neurological and immune development (gut-brain-immune axis). Therefore, our objective is to evaluate the effect of iron supplementation on the composition of the gut microbiome in 5-month-old infants.

**Methods:** The study will be of a cross-sectional observational type. Participants will be recruited from primary care centers in the city of Arequipa, and 2 study groups will be formed with 30 participants each: 1) Infants with 5 months of age (I5M) who have received ferrous sulfate 2 mg/kg/day at 4 months for at least 1 month, 2) I5M who have not received ferrous sulfate at 4 months. During the monthly medical check-up of the infant, sociodemographic information will be collected, anthropometric measurements will be taken and peripheral venous blood will be collected to quantify the levels of hemoglobin, ferritin, transferrin, hepcidin, and C-reactive protein. Stool samples will also be collected for analysis of the gastrointestinal microbiome for the extraction of microbial genetic material, purification, amplification, and subsequent massive sequencing of 16S ribosomal RNA. Bioinformatic analysis and statistical analysis will be performed using the QIIME pipeline and R software. Relative abundance, alpha, and beta diversity are compared using Welch's t-test or Wilcoxon's rank-sum test depending on the normality of the data. A multivariate analysis will be carried out according to general linear models. The study will be carried out after approval by a certified ethics committee. We expect to find a significantly higher abundance of the *Clostridium* and *Escherichia/Shigella* genera and a lower abundance of the *Bifidobacterium* and *Lactobacillus* genera in 5-month-old infants receiving iron supplements.

**Key Words:** Anemia, Iron Deficiency, Gut Microbiome

### III.9.4. Contribution of CXCR1 in the generation of enriched sphere cultures in cancer stem cells derived from ovarian cancer cells.

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**Introduction:** Worldwide, ovarian cancer is one of the ten leading causes of cancer-associated death among women. In Perú, around 2.0% of cancer mortality is related to this type of tumor. There is evidence that a small population of cells within tumors called cancer stem cells (CSCs) would be responsible for treatment failure due to their greater ability to initiate metastasis and acquire chemoresistance. One of the properties of these cells is the capacity for self-renewal, which is promoted by the activation of different signaling pathways, including the interleukin 8 (IL-8) / CXCR1 (CXC Motif Chemokine Receptor 1) pathway, which is a fundamental modulator of the self-renewal and differentiation activity of most CSCs, such as epithelial-mesenchymal transition and metastasis. The general objective of the present investigation is to evaluate the contribution of CXCR1 in the generation of cultures of spheres derived from HeyA8 ovarian cancer cells with traits of cancer stem cells.

**Methodology:** i) Lentiviral particles that encode an interference RNA for the silencing of CXCR1 were designed and generated in the HeyA8 cell line (lentiviral transduction); ii) the contribution of CXCR1 in the formation of ovarian cancer cell spheres was evaluated (spheres formation assay); and iii) the gene expression of tumor stem cell markers (SOX2, NANOG, and Oct4) in the spheres formed was evaluated.

**Results:** The silencing of CXCR1 (HeyA8<sup>shCXCR1</sup>) and the control (HeyA8<sup>shLuc</sup>) were corroborated by flow cytometry. A statistically significant decrease was observed in the diameter and number of tumorspheres derived from HeyA8<sup>shCXCR1</sup> cells with and without IL-8 compared to the HeyA8<sup>shLuc</sup> control with IL-8. Conventional PCR assays indicated a decrease in the gene expression of NANOG, SOX2, and Oct4, markers of pluripotentiality, in spheres derived from HeyA8<sup>shCXCR1</sup> cells with and without IL-8 compared to those derived from HeyA8<sup>shLuc</sup> cells with IL-8, although a slight increase was observed compared to the HeyA8<sup>shLuc</sup> control without IL-8.

**Conclusions:** Our results show that the activation of CXCR1 contributes to the formation of spheres derived from HeyA8 ovarian cancer cells, enriching this type of culture with cancer stem-like cells. These results suggest that CXCR1 would favor the proliferation of these cells in ovarian cancer, which are associated with different pro-tumorigenic properties such as increased chemoresistance and the induction of metastasis.

**Key Words:** Ovarian cancer, cancer stem cells, tumorspheres, CXCR1.

**III.10.1. Thermographic imaging accuracy in the diagnosis of infected diabetic ulcer in the context of primary healthcare.**

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**Introduction:** Clinical diagnosis of diabetic ulcer (DU) infection is performed according to the Infectious Diseases Society of America (IDSA). Infrared thermography is an innovative noninvasive biomedical imaging modality for diagnostic support of the diabetic foot. The objective was to determine the diagnostic accuracy of thermographic imaging (TI) concerning clinical diagnosis by IDSA in DU with suspected infection in an outpatient setting.

**Methodology:** The study design was diagnostic tests. The unit of analysis was the DU located in each foot. Diabetic patients were recruited in primary healthcare (PHC) centers for one year. Epidemiological and clinical data collection was by interrogation and neurovascular examination of the foot. The diagnosis of clinical infection according to IDSA was considered when 3 or more symptoms and signs such as pain, redness, purulent discharge or cellulitis, fasciitis, abscess, or osteomyelitis were present. The IT collection was performed using a FLIR E8 camera after adaptation. The processing of the captured TI was carried out by FLIR TOOL software performing intralesional (IL) and extralesional (EL) sectorial and punctual thermographic measurements according to the algorithm by Spahn. The value of the difference between the lowest sectorial EL measurement minus the lowest punctual IL was considered as thermographic infection (3°C or more).

**Results:** The DU evaluated with suspected infection were 80 and their clinical characteristics were length and width in  $2.47 \pm 2.14$  cm and  $1.93 \pm 1.43$  cm respectively; the diagnosis of clinical infection according to IDSA was 56.25%. The characteristics of the TI were sectorial IL and EL  $31.27 \pm 2.26^\circ\text{C}$  and  $33.04 \pm 2.16^\circ\text{C}$  respectively, the diagnosis of IT infection was 53.75%. The calculation of accuracy (A) is the average of the sum value of sensitivity (Se) and specificity (Sp); Se was true positives/true and false positives, Sp was true negatives/true and false negatives. The Se, Sp, A was 82%, 100%, and 91% respectively.

**Conclusion:** This original study of diagnostic tests in 80 DU with suspected infection using IT test concerning the standard had a very high A. This implies that this image can be used for the early detection of infection in DU.

**Key Words:** diagnostic accuracy, thermographic imaging ,diabetic ulcer, infection.

### III.11.1. Comparative study of lymphocyte subpopulations and cytokine profile in blood of patients with COVID-19 and active tuberculosis.

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**Introduction:** Perú is one of the countries most affected by COVID-19 worldwide. In addition, Perú is endemic in tuberculosis (TB), which predisposes to a high probability of coinfection that could worsen the prognosis of the disease. Although both diseases share certain immune response mechanisms, evidence is scarce and comes from non-endemic countries.

**Objective:** To compare lymphocyte subpopulations and cytokine profile between patients with COVID-19/TB-active coinfection and patients with COVID-19, TB-active, and without COVID-19 or TB-active.

**Methods:** Prospective observational cohort study. It will be performed in the COVID-19 hospitalization services of 2 hospitals in Arequipa during the second semester of 2021. There will be 4 study groups (n=15) composed of patients with COVID-19/TB-active, COVID-19, TB-active, and without COVID-19 or TB-active. On the first and seventh day of hospitalization, blood serum cytokine levels (IL-1 $\beta$ , IL-6, IL-8, IL-8, IL-10, IL-12, IL-17, INF- $\gamma$ , TNF- $\alpha$ ) by ELISA and Peripheral Blood Mononuclear Cells (PBMC's) will be isolated to identify activation and memory markers (CD3, CD4, CD8, CD45RA, CCR7, CD27, CD28, IgD, CD38, CD95, CD14, CD56, CD19) on T, B and NK lymphocytes by flow cytometry. Statistical analysis will be performed in R, we will explore the distribution of the data and use parametric or non-parametric tests to compare groups as appropriate. The analysis will be performed in FlowJo10 software to analyze high-dimensional data obtained from flow cytometry. All procedures will be performed after approval by an accredited ethics committee.

**Expected results:** In patients with COVID-19/TB-active coinfection, serum levels of IL-1, IL-2, IL-6, IL-8, IL-8, IL-10, IL-12, IL-17, INF- $\gamma$ , and TNF- $\alpha$  and the immunophenotype of circulating CD4, T CD8, B, and NK lymphocytes will differ significantly compared to patients with only COVID-19 or TBa diagnosis. In addition, we expect to find a decreased Treg lymphocyte response and a marked Th1 and Th17 response, causing further immunopathological damage and early development of cytokine storm.

**Key Words:** Tuberculosis, COVID-19, cytokines, lymphocytes.



### III.11.2. Effect of nafamostat and bromhexine on genes related to viral entry and ACE2 in hACE2 transgenic mice exposed to recombinant spike protein from SARS-CoV-2

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**-Introduction:** The current treatment against COVID-19 is symptomatic and supportive, there is still no conclusive evidence to support the use of antiviral drugs as a therapeutic weapon. Despite knowing the viral entry mechanisms of SARS-CoV-2, it is crucial to be able to understand other mechanisms that favor the infection process. Nafamostat and bromhexine were shown to inhibit TMPRSS2, a protein involved in the cellular entry of SARS-CoV-2 after binding to the ACE2 receptor. Therefore, the objective of the present project is to evaluate the effect of nafamostat and bromhexine on genes related to ACE2 and viral entry in K18-hACE2 transgenic mice exposed to the recombinant SPIKE protein of SARS-CoV-2.

**-Methodology:** For the experimental design, K18-hACE2 (mK18-hACE2) mice will be exposed intratracheally to the recombinant Spike protein of SARS-CoV-2 (RSP). The 2 proposed drugs will be applied intraperitoneally 2 hours before exposure to RSP. 7 study groups will be made up of 5 mice each: T1 (wild-type mice), T2 (wild-type mice+RSP), T3 (mK18-hACE2), T4 (mK18-hACE2+RSP+nafamostat), T5 (mK18-hACE2+RSP+bromhexine), T6 (mK18-hACE2+RSP+nafamostat+bromhexine), T7 (mK18-hACE2+RSP). After 48 hours post-intratracheal exposure to RSP, the mice will be euthanized and the corresponding samples of lung tissue and upper respiratory tract will be extracted. They will be stored and frozen for transfer, complying with the storage and cold chain protocols for further processing. We will study the genes involved in viral entry (ACE2, TMPRSS2, TMPRSS4, CTSB, CTSL) and the genes associated with the expression of ACE2 (CAV2, LC3B, LAMP1, ITGB6, LY6D, IFITM3). For the study of gene expression, the process of quantitative reverse transcription polymerase chain reaction (RT-qPCR) will be carried out in real-time and for the analysis of protein expression we will carry out the Western Blot method. The data obtained will be emptied into a Microsoft Excel spreadsheet to perform the statistical analysis in the R software. The data will be compared using the ANOVA or Kruskal-Wallis test. A p-value <0.05 will be considered significant. We expect that the expression of the genes involved in viral entry and associated with the expression of ACE2 will be decreased in the groups that received nafamostat and/or bromhexine.

**Key Words:** SARS-CoV-2, TMPRSS2, nafamostat, bromhexine

### III.11.3. Analysis of tissue and viral entry proteins in hACE2 transgenic mice exposed to recombinant spike protein treated with nafamostat and bromhexine

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**-Introduction:** The alarming lethality of the COVID-19 pandemic continues as new variants of interest and concern have emerged in the public health arena. Within this context, the appearance of a considerable variety of vaccines stands out, which have shown to have different efficiencies and effectiveness. On the other hand, different clinical trials were conducted looking for a drug against SARS-CoV-2. However, an effective treatment has not yet been found, so the urgency to find a therapy and / or prophylaxis other than vaccination remains, especially in countries where vaccination progresses slowly. Nafamostat mesylate is an inhibitory anticoagulant of TMPRSS2, which plays a crucial role for the entry of the virus. Currently, Japan will initiate a clinical trial for the combination treatment of nafamostat mesylate and favipiravir, as a previous study showed efficacy in critically ill patients. Likewise, bromhexine hydrochloride has a randomized controlled pilot study, where it showed efficacy in patients with mild or moderate disease. However, both drugs require definitive large-scale clinical trials and their use as prophylactics requires critical evaluation. In order to understand the molecular mechanism of both drugs, biomarkers must be found and standardized. To do this, we seek to evaluate the effect of nafamostat mesylate and bromhexine hydrochloride on the tissue proteomic profile and viral entry in K18-hACE2 transgenic mice exposed to the recombinant spike protein treated with the mentioned drugs.

**-Methodology:** With prior approval by an Ethics Committee, the murine models will be exposed to the recombinant spike protein, establishing a control group and three experimental groups, in which nafamostat mesylate and / or bromhexine hydrochloride will be administered; then pre and post treatment comparisons will be made. The administration of the drugs will take place two hours before exposure to the spike protein. After 24-48 hours, the murine models will be euthanized, followed by extraction and storage of the lungs at -40 ° C until gel digestion, followed by proteomic analysis using a RP-HPLC/MS/MS. Once the differentially expressed proteins have been identified, a quantitative Western Blot will be performed.

**-Results:** It is possible that the marker proteins of the SARS-CoV-2 viral entry show a differential expression in favor of an antiviral state, while the tissue marker proteins could reduce their expression as is the case of TMPRSS2 or increase the expression of proteins related to phagocytic activity.

**-Conclusions:** The study will provide insight into the molecular mechanism of nafamostat mesylate and bromhexine chloride as potential candidates for the treatment or prophylaxis of COVID-19, as well as a further rationale for initiating meaningful clinical trials.

**Key Words:** COVID-19, Nafamostat, Bromhexine, Proteomics

### III.12.1. Molecular characterization of lactic acid bacteria and protozoa *Eimeria* sp., from the gastrointestinal microbiota of *Gallus gallus domesticus*

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**-Introduction:** The knowledge of the chicken microbiota, *Gallus gallus domesticus*, is essential in poultry production, in physiological, zootechnical and veterinary terms. Worldwide, studies are presented as a priority in the poultry sector with research focused on probiotic and/or pathogenic microorganisms.

**-Methodology:** Bacterial strains isolated from the different gastrointestinal compartments of the chicken were molecularly characterized using the PCR technique (Polymerase Chain Reaction), with which in vitro tests were performed such as resistance to acidic pH, resistance to bile and tests of antagonism against *Salmonella typhimurium*. Likewise, bacterial strains and parasites *Eimeria* sp. Were molecularly characterized using the MALDI-TOF-TOF double mass spectrometry technique.

**-Results:** 32 bacterial strains were isolated, the partial sequencing of the 16S rRNA gene allowed the identification of eight lactic acid bacterial strains from the ventricle (*Weissella* sp.), The small intestine (*Lactobacillus brevis*, *Lactobacillus farciminis*, *Lactobacillus plantarum*, *Lactobacillus pentosus*) and of the large intestine (*Pediococcus pentosaceos*, *Enterococcus hirae* and *Enterococcus faecium*). The bacterial strains resisted to acidic pH (2.5, 3.5 and 5.0) and to bile (0.10, 0.15 and 0.30), they showed inhibition halos against *Salmonella typhimurium*. Lactic acid bacteria were also identified by means of their proteins, as well as seven species of *Eimeria* sp., Based on the peptide fingerprint of previously purified oocysts in a Percoll density gradient. A total of 41 proteins were identified for bacterial strains and 42 proteins for protozoa of the genus *Eimeria*.

**-Conclusions:** These results will contribute to the availability of new probiotic strains for the poultry sector and to the modernization of the technologies for characterization and molecular identification of microbiota microorganisms

**Keywords:** 16S rRNA gene, lactic bacteria, *Eimeria* spp, Mass spectrometry

### III.12.2. Uranyl salen-type complex as cocatalyst for electrocatalytic oxidation of ethanol

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**Introduction:** Among the advanced energy technologies, Direct ethanol fuel cells (DEFC) have been considered a promising alternative due to their low environmental impact. However, it is difficult to cleavage the ethanol C–C bond to generate CO<sub>2</sub> and H<sub>2</sub>O. To date, Pt is one of the most effective metals used in it. However, pure Pt is not effective due to the poisoning of COads intermediates. Therefore, in recent decades, a wide range of approaches have been employed to improve the electrocatalytic performance of Pt-based electrocatalysts. Reports on the literature deal with salen-type uranyl complexes with diverse applications, such as receptors, carriers, sensors, and also biological activities. The present work presents the application of a Schiff base uranyl complex, [UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O], as modifier of platinum/tin-based electrodes for the electrooxidation of ethanol.

**Methodology:** Preparation of uranium(VI) complex [UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] and characterization was performed by physical measurements and spectroscopic analysis. Simultaneously the metallic catalyst PtSn/C was prepared by the formic acid method. Then, the electrochemical activities of PtSn/C and PtSn/C:[UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] catalysts at different mass proportions of PtSn/C and [UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] (6:1; 5:1 4:1; 3:1 and 2:1) were measured against the electrooxidation of ethanol within the potential range 0.05 - 1.1 V from cyclic voltammetry and chronoamperometry analysis.

**Results:** The start of ethanol electrooxidation for the 6:1 PtSn/C:[UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] electrocatalysts was approximately 0.29 V, while for the PtSn/C and mixed electrocatalysts 5:1; 4:1; 3:1 and 2:1 PtSn/C:[UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] the potential was positively displaced. Among the mixed catalysts, within the potential range of interest for DEFC (0.2-0.6 V), the current for the 6:1 electrocatalysts was higher than all the others mixed electrocatalysts shown in this work. This result was confirmed by chronoamperometry. The peak current density for the mixed catalyst 6:1 is 32.93 mA cm<sup>-2</sup>, that is, ~4.8 times that of PtSn/C, which results in a more favorable reaction kinetics for alcohol oxidation.

**Conclusion:** Herein, a uranyl complex containing a ONNO Schiff base ligand 3-OMe-*t*-salcn has been used as cocatalyst for Electrocatalytic Oxidation of Ethanol. Preliminary results indicated that PtSn/C:[UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] at 6:1 mass ratio promotes better catalytic activity for ethanol electrooxidation than that of PtSn/C and [UO<sub>2</sub>(3-OMe-*t*-salcn)H<sub>2</sub>O] alone.

**Keywords:** Uranyl complexes; Schiff base; Fuel Cell, Advanced Electrooxidation.

### III.12.3. Cloning, expression and purification of septins and botulinum neurotoxin serotype A (BoNT/A)

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#### -Introduction:

Septins are organized into filaments, networks, or rings to form part of cytoskeletal proteins. In functional terms, septins are related to various cellular processes, from cell division, the formation of physical barriers, diffusion, and also the binding of proteins to substrates, such as the plasma membrane. Botulinum neurotoxins (BoNT) are potent toxins with protease activity that cleave SNARE proteins and cause flaccid paralysis, leading to severe symptoms of human neuroparalytic botulism, with botulinum toxin serotype A having more prolonged effects.

#### -Methodology:

The coding sequences of the target human septins, as well as the recombinant expression constructs are available from the Molecular Biophysics Group of the IFSC of the University of Sao Paulo. Access to the coding sequences of LCA and mutant LCA were obtained commercially, through synthetic genes. All DNA cloning, subcloning and manipulation procedures in general were carried out according to the standard molecular cloning techniques. Protein expression was carried out in *E. coli* strains BL21 (DE3) and / or Rosetta. (DE3). Expression occurred after the addition of the inducing agent (IPTG) in the cultures, which were harvested by centrifugation and lysed by ultrasound. The soluble and insoluble protein fractions were separated by centrifugation and the soluble fraction was subjected by affinity chromatography (according to the recombinant label). Size exclusion chromatography (Superdex 200 or Superose 300) was then used to separate the complexes of interest from the aggregates.

#### -Results and Conclusions:

In this context, understanding the role of molecular characteristics in the interaction between LCA and human septins will help to clarify the mode of action and stabilization of toxins, also allowing the development of non-procedural intervention strategies aimed at new therapies.

**Key Words:** cloning, BoNT/A, septins, cytoskeleton



### III.12.4. GC-SM of bioactive compounds of *Satureja incana*'s essential oil

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**Introduction:** *Satureja incana* is an endemic species of Perú, used in ethnomedicine as: béquico, carminative, antispasmodic in high Andean communities by way of infusion of the flowery tops, in the Chiuchepata area at 2682 meters above sea level in the district of Palca, Tarma Province, Junin Region. The present study aims to: Identify and quantify the composition of phyto-bioactives present in the essential oil of *Satureja incana* by gas chromatography coupled to mass spectrometry (GC-MS); determine extractive performance and the physical-chemical characterization of the essential oil.

**Method:** Essential oil extracting using a stainless-steel extraction equipment, extraction time 1 hour, open evaporation system; and, analytical method with GC-SM: flow helium gas 20 mL.min<sup>-1</sup>, injection of 0.2 µL of essential oil, thermal configuration in gradient.

**Results:** essential oil extraction yield of 0.49% in the tender branches in the flowering stage, with a cut of the top of 20-35 cm; relative density 0.9816; refractive index 1.4879; and the phytochemical composition reported by GC-SM indicates that a 66.37% sesquiterpenic concentration and 30.07% monoterpenes predominate; being the main compounds: germacrene D 25.91%, β caryophyllene 22.10%, α -cymene 12.62%, 4 (8) -p-menthone 6.73%, humulene 3.95%, caryophyllene oxide 3, 08%, limonene 2.44%; and minority: β -bourbene 1.95%, β-ocimene 1.78%, spatulenol 1.66%, linalol 1.64%, isopulegol 1.66%, α-cubebene 1.51%, 5-Cadinene 0, 89%, α-pinene 0.45%, β-pinene 0.52%.

**Conclusion:** The presence of some chemical compounds supports their ethnobotanical use by high Andean populations. It has the potential to be used in pesticide formulations due to the presence of germacrene D in high concentration.

**Key Words:** Ethnobotanical, essential oil, *Satureja incana*, GC-SM.

### III.12.5. Molecular characterization of lectins from seeds of *Sambucus nigra* L. (Elder) and their biotechnological applications.

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**Introduction:** In the present research work, the function of a lectin from *Sambucus nigra* L. seeds was isolated and molecularly characterized, purification was carried out by Sephadex G-75 molecular exclusion chromatography, and haemagglutination tests were performed. The haemagglutinating fraction was purified by HPLC-FR chromatography; Thus, the lectin isolated from *S. nigra* L was also physically-chemically characterized. Through amino acid composition analysis, it was possible to determine the molecular mass of the fraction (hemagglutinating fraction) of 30,612 kDa by SDS-PAGE electrophoresis. The amino acid composition analysis revealed an acidic protein (25.75% acid residues). The study of the hemagglutinating activity of the SamL lectin showed that it is capable of agglutinating blood types "A", "B" and "AB", Presenting a selectivity for groups "A", "B" and "AB" Rh (+) at a minimum concentration of 7.5 µg/mL. In this way we can conclude that SamL belongs to the group of mannose binding lectins. Finally, the SamL lectin caused the inhibition in the fungi *Fusarium solani* and *Aspergillus niger* and also exhibited antitumor activity on the growth of SW480 cells of Human Colon Adenocarcinoma.

**Methodology:** The mature seeds used belong to *S. nigra* L. from the city of Cusco-Cusco region. The crude extract was obtained from the seeds of *S. nigra* L., using monobasic sodium phosphate as a buffer, 1 M pH 7.6 for two hours under continuous stirring. The homogenate obtained is centrifuged at 6000 g for 30 minutes. Next, a supernatant is obtained, named crude extract (EC), it is dialyzed with distilled water for 48 hours, then the hemagglutination and carbohydrate inhibition test is applied. We proceeded to use sephadex G-75 molecular exclusion chromatography purification and also using HPLC-FR chromatography and complementing the amino acid analysis was an automatic PICO TAG amino acid analyzer (Waters System), where the determination of the amino acids is performed through HPLC chromatography of the phenylthiocarbamyl product of the amino acid and finally a one-dimensional electrophoresis was carried out. Finally, with the purified fraction, hemagglutination analyzes were carried out with the red blood cells and the biological analyzes and the antifungal effects and viability tests with the cancer cells were verified.

**RESULTS:** A lectin (SamL) was isolated from seeds of *S. nigra* L. using two chromatographic steps: molecular exclusion chromatography on Sephadex G-75 and reverse phase high precision chromatography (RP-HPLC). Then, by electrophoresis it was determined that the SamLec lectin from *Sambucus nigra* L. it has a molecular mass of 30.61 kDa revealed a protein of monomeric nature of 30,612 Da. Which was supplemented with automatic amino acid analysis showed a high content of acidic amino acids. And finally, SamL lectin was able to agglutinate group "A", "B" and "AB" Rh (+) erythrocytes at a minimum concentration of 7.5 µg/mL. The SamL lectin presented inhibitory activity in vitro on the growth of *Fusarium solani* and *Aspergillus niger* with a concentration of 100 µg/mL, thus inhibitory activity was also obtained in vitro on the growth of SW480 cells of Human Colon Adenocarcinoma.

**Key Words:** Lectins, proteins, cellular viability, antifungal.

**III.13.1. Mercury and sediment content of the Azangaro river and its effect on the people of progress****Eliana Mullisaca Contreras**

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E-mail: [e.mullisaca@unaj.edu.pe](mailto:e.mullisaca@unaj.edu.pe)**ABSTRACT**

The mining seats, present in the upper part of the Ramis river basin, such as: La Rinconada, Lunar de Oro, Pampa Blanca, Ananea and Crucero when dumping their mining tailings into the river mainly composed of mercury (heavy metal used in the process of amalgamation of gold), causes its contamination in the Azángaro river; the same one that is used by the inhabitants of the surrounding districts such as Asillo, Progreso and Azángaro for their consumption, agriculture and livestock, the inhabitants being affected by consuming these contaminated waters, the objective was to evaluate the contamination of the Azángaro river and its effect on The inhabitants of the town of Progreso, water and sediment were sampled at three points: M1, M2 and M3, taking into account the regulations, it was transported to the Environmental Quality control laboratories of the Universidad Mayor de San Andrés de la Paz-Bolivia with its respective checklist. The methodology used in the determination was the atomic fluorescence method-EPA 205. The results showed that the Azángaro River presents concentrations of mercury equal to 0.00020 mg/L, it is below the limits given by the ECA (0.001 mg/L); in the case of sediments, they presented values equal to 1.5; 0.20 and 0.20 mg/Kg in M1, M2 and M3, being higher than those given by USEPA (0.15). In conclusion, the mercury concentration is below the limits given by the ECA, presenting no apparent contamination; however, mercury concentrations were found in sediments higher than USEPA.

**Key Words:** Mercury, mining, Azángaro river, sediment

## **Index by authors**

A. Camacho Crespo,40  
A.T. Lombardi,67  
Adolfo Hernández,28  
Adolfo Ramos Paredes,100  
Alberth Ticona,57  
Alcibiades Helí Miranda Chávez,44  
Alejandro Fukusaki-Yoshizawa,89  
Alexandre Campos,48,53  
Alfredo Torres-Benítez,81  
Allshine Chen,24  
Álvaro Modernell,26  
Amanda Ellen Lima,66  
Ana C. Fonseca,74  
Ana Freire da Silva,73  
Ana Mayanga-Herrera,89  
Ana P. Mucha,48  
Ana Paula Ulian de Araujo,98  
Ana Rita Rodrigues Favas,49  
Ana Teresa Lombardi,30,66,75,87  
André Melo,29  
Andrea Diestra,23  
Andrés González-Techera,26  
Andreza Miranda Barata da Silva,79  
Angélica Ysabel Miranda Jara,44  
Anish Babu,24  
Anne Boland,17  
Annette N. Trombert,94  
Annette Trombert,28,69,70  
Anthony Fuentes Olivera,11

Anthony Idone Accha,82,83,84,85  
Antonio Lazarte Rivera,59, 61,69,70,82,83,84,85,94,95  
Anupama Munshi,24  
Armando González Sánchez,16,56,57  
Bernard Marte,37  
Betsy Rosemary Quispe Cruz,54  
Bing Yu Zou,25  
Blitza E. Yauri Villanueva,94  
Brando Ortiz-Saavedra,90,93,94  
C. Chacón,18  
C. Marisa R. Almeida,48  
Camila Castro,12  
Candelario Vázquez-Cruz,22  
Carla Palomino-Durand,37  
Carlos A. Arenas Chavez,88  
Carlos Ballon-Salcedo,95  
Carlos Elias Tirado Rodriguez,58  
Carlos Padilla Rojas,35  
Carlos Pariona-Velarde,65  
Carlos Riquelme,43,52  
Carlos Scotto,38  
Carlos Tong-Rios,25  
Carmen Rosario Yauri Chacón,69,70  
Catarina Gonçalves,55  
Catarina Magalhaes,48  
Cédric Delattre,72  
Cesar de la Fuente,5  
Cesar Ivan Mejia Llontop,58  
Cesar Ramal,23  
Cesar Ranilla,57



Christian Sánchez,68  
Christopher Fitzpatrick,17  
Claudia M. Gonzales Garcia,94  
Claudio Alejandro Sáez Avaria,13  
Claudio Villota,17  
Constanza Cárdenas,45  
Cristina Sousa Coutinho Calheiros,14  
Cristofer Chambi,57  
Daladier Castillo Cotrina,63  
Daniel Andrade,24  
Daniel Angel Sanchez Vaca,58  
Daniel de los Cobos-Vasconcelos,56,57  
Daniela Arequipa Loayza,59  
Dante Rodríguez Alonso,92  
David Urquizo Valdivia,82,83,84,85  
Diego B. Menezes,32  
Diego Pérez-Stuardo,3  
Diogo A.M. Alexandrino,48  
Diogo Cruz,72  
Dionicia Gamboa,25  
Dolores Bautista-España,22  
Dominique Fernandes de Moura do Carmo,78,79  
E. Miahle,96  
Eberlanny Moraes Rolim,79  
Edgar Chaparro Aguilar,63  
Edgar Ttito Quispe,82,83,84,85  
Edgardo R. Donati,12  
Eduardo Perochena-Escalante,50,65  
Edwin Bocardo Delgado,46  
Edwin Ccoyllo-Terrones,71

Elena A. Vidal,3,  
Eliana Mullisaca Contreras,101  
Elizabeth A. Winzeler,25  
Elizomar Medeiros Barbosaa,97  
Eloy Condori Mamani,98  
Elson Almeida Souza,97  
Emma Paytas,25  
Eric Barrey,17  
Estela Anastacio-Marcelino,22  
Eva Vallejos-Vidal,3  
F. Fabian,96  
Felipe E. Reyes-Lopez,3,  
Félix Pompeyo Ferro Mayhua,39  
Feng Chai,37  
Flávio Augusto de Freitas,79  
Francisca Parada,9  
Franco Simini,34  
Franklin Vargas,68  
Frédéric Cazaux,37  
Froy Engelbert Coloma Dongo,86  
Gabriel Lassabe,26  
Gabriela Moreira,29  
Gabriele Simas Ribeiro,77  
Gino Nardocci,17  
Graciliana Lopes,49  
Graciliana Lopes,51  
Greg LaMonte,25  
Gualberto González-Sapienza,26  
Guillaume Pierre,72  
Gustavo Frigi Perotti,77,78,79

Hannah Steinberg,23  
Harold Renzo Chirinos Urday,46  
Harrison Montejo Arevalo,35  
Hector Alvarez-Vega,89  
Henri Bailon Calderon,35  
Henry Cameron,52  
Hugo Osório,48  
Ingrid Lucero Cacya Apaza,88  
J. Andrés Rivas-Pardo,3  
J.C. Silva,67  
Jacqueline Estefany Aza Suaña,62  
Jaeson Calla,25  
Jaime Villegas,17  
James Griffith,24  
Janaína Morone,49,51  
Jaqueline Carmo Silva,66  
Javier Montalvo,57  
Javier Montalvo-Andía,56  
Javier Paredes-Mella,4  
Jazmín Villanueva-Aronés,50  
Jean-François Deleuze,17  
Jefferson Bustamante Rivera,60  
Jeffrey Aube,24  
Joana Azevedo,48,53  
Joana R. Almeida,55  
João Morais,73  
Jorge Bendezu Eguis,21  
Jorge Gonzales,95,19  
Jorge I. Mardones,4  
Jorge Parodi,10

Jorge Valdés,52,59  
Jose Amiel-Pérez,89  
José C. Cheel Horna;,88  
José Fernando Oñate Garzón,60  
José González,68  
José Laura Huamán,82,83,84,85  
José Luis Maturana,27  
José R. V. Baudrit,32  
José Wilmo da Cruz Jr,97  
Josefina Plaza Cazón,12  
Joseph M. Vinetz,25  
Joseph Obed Ricaldi Sarapura,99  
Joshua Corbin,24  
Juan Antonio Sanpedro-Luna,22  
Juan Lévano Saravia,35  
Juan P. Cárdenas,27  
Juan San Francisco,19  
K Macedo Rodney,93  
Kaique Soares Souza,97  
Kathleen Abt,29  
Kenyi Arango Farias,82,83,84,85  
Kevin Flores-Lovon,93  
Kyoko Yarimizu,4  
L. Lizama,18  
L. Vazquez,96  
L.A. Quispe-Cueva,93  
Lalo Monzón-Martínez,56  
Laura María Isabel López,8  
Lauren Stadler,36  
Layné Guerra,25

Leonardo de Faria,87  
Leonel González,52  
Leonor Ferreira,55  
Liang Xu,24  
Licia Salvatierra-Capcha,50,65  
Lindomar Cordeiro Antunes de Araújo,77,80  
Liseth Rossela Orosco Barrionuevo,91  
Lizbet Susan Montes-Madariaga,93  
Lorena Lobos-Gonzalez,17  
Lucas Gentilini,20  
Lucas Santos Solidade,87  
Luis Felipe Valdez Núñez,54  
Luis O. Burzio,17  
Luis Pereira da Costa,79  
Luis Vilcahuaman,33  
Luiz P. da Costa,32  
Luiz Pereira da Costa,77,78,80,97  
M. Baylon,96  
Macarena Briones,17  
Manuel Varas-Godoy,91  
Marco Lopez,37  
Marco Preto,29,51  
Margarida Carmo de Souza,80  
Margith Romero,57  
Maria Elena Suaña Quispe,47  
Maria F. Carvalho,48,74  
Maria Lucila Hernández-Macedo,32  
María Patricia Sánchez-Alonso,22  
María Paz Covarrubias,42  
Maria Quenta,61



María Sofía Urbieto,12  
Maria Teresa Mata,43,52  
Mariana Alves Reis,76  
Mariana Reis,55,74  
Mario Esparza Mantilla,31,60  
Mario Simirgiotis,7,81  
Maritza Calderón,23  
Marlene Pinto,45  
Martín Montecino,17  
Martina Scarrone,26  
Mathias Flores-González,81  
Matthew Ykema,36  
Maximiliano F. Bendek,17  
Meghna Mehta,24  
Michael Handford,41,42,6,82,83,84,85  
Miguel Semedo,48  
Myriam Gorospe,24  
N.I Valdez,67  
Naomi Senehi,36  
Natália Gonçalves Silva,76  
Natalia Montellano Duran,40  
Nicolas Blanchemain,37  
Nicole Farfán,17  
Ofelia Córdoba,68  
Omar Cáceres Rey,35  
Orquídea Merma Torres,59  
P. Masias,96  
Pablo Berríos,28  
Pamela Cabedo,41  
Patricia Chocano-Bedoya,93

Paulo José de Sousa Maia,79  
Paulo José Sousa Maia,97  
Pedro Alvarez,61  
Pedro J.J. Alvarez,36  
Pedro N. Leão,29  
Pedro Valencia,45  
Philippe Michaud,72  
Pingfeng Yu,15  
Rachid Harba,33  
Rajagopal Ramesh,24  
Ralf Janknecht,24  
Ralph Urbatzka,2,74,76  
Raphael Canals,33  
Renata Porto Sampaio,75  
Rita Mendes,53  
Robert Gilman,23  
Roberto Vega Paulino,11  
Romina Álvarez-Rosado,26  
Ronald Huarachi Olivera,43,82,83,84,85,52,70,94,95  
Rosa Atayupanqui-Dueñas,56  
Rosário Martins,51,49  
Roxana Bardales Álvarez,86  
Ruonan Sun,36  
S. Ampuero,18  
Salyoc Tapia-Rojas,89  
Sanam Husain,24  
Sandra A.C. Figueiredo,29  
Sandra Pereira,55  
Sangphil Oh,24  
Santiago Benites Castillo,92

Satoshi Nagai,4  
Sebastián Caro-Retamozo,71  
Sebastián Reyes-Cerpa,3,  
Sergio Almonacid,45  
Shalom Apérrigue-Lira,93  
Sidney Souza dos Santos,78  
Silvia Capristano Valdez,35  
Sirje Sildever,4  
Sudeshna Roy,24  
Suleivys M. Nuñez,45  
Supriyo De,24  
Susana Sirvas-Cornejo,50,65  
Terence Herman,24  
Teresa P. Martins,29  
Thiago Lourenço Gomes,80  
Tiago Guimarães, 64  
Tomé Azevedo,53  
Triana Delfin-Riela,26  
U. Urzúa,18  
Valeria A. Silva,17  
Vanesa Buleje-Alfaro,50,65  
Vannia Alessandra Salas Loayza,69,70  
Verónica A. Burzio,17  
Verónica Silva,17  
Verónica Yaniro Coronel,35  
Vincenzo Borgna,17  
Vitor M. Vasconcelos,29  
Vitor Vasconcelos,1,49,51,53,55,64,72,73,74,76  
Weiwei Caod,48  
William Paucar Quispe,11

Wilmer Paredes Fernández,100

Yan D. Zhao,24

Yhon Saico Pallani,100

Yizhi Tao,36

Zhamanda N. Ortiz Benique,90

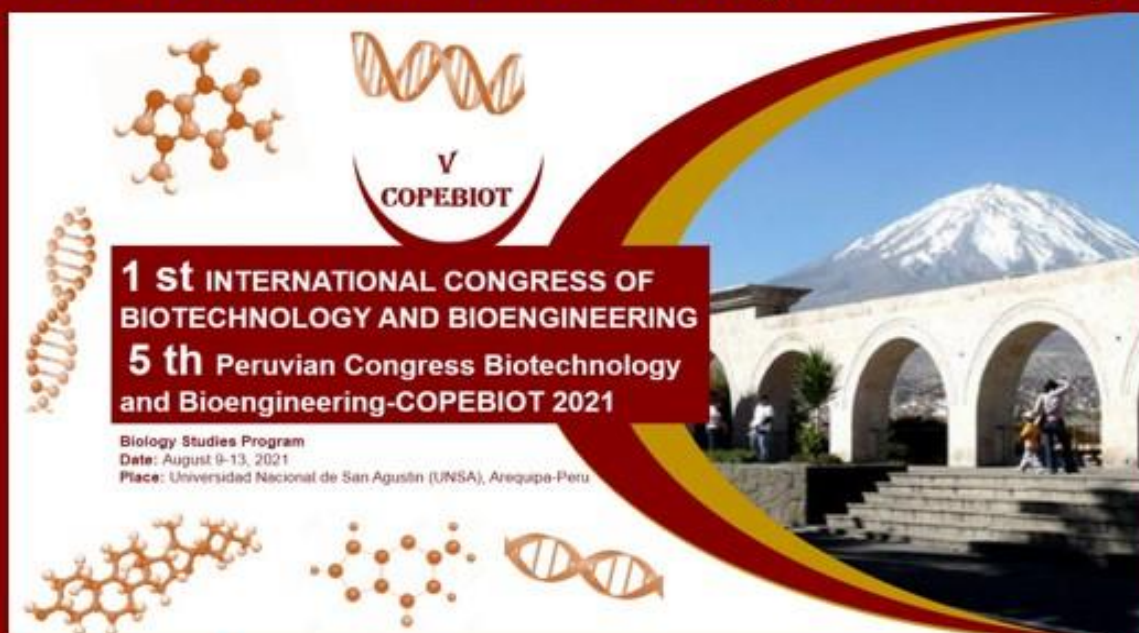
Zhongjun Hia,48

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# "Year of the Bicentennial of Peru: 200 years of Independence"



**1<sup>st</sup> INTERNATIONAL CONGRESS OF BIOTECHNOLOGY AND BIOENGINEERING**  
**5<sup>th</sup> Peruvian Congress Biotechnology and Bioengineering-COPEBIOT 2021**

Biology Studies Program  
Date: August 9-13, 2021  
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