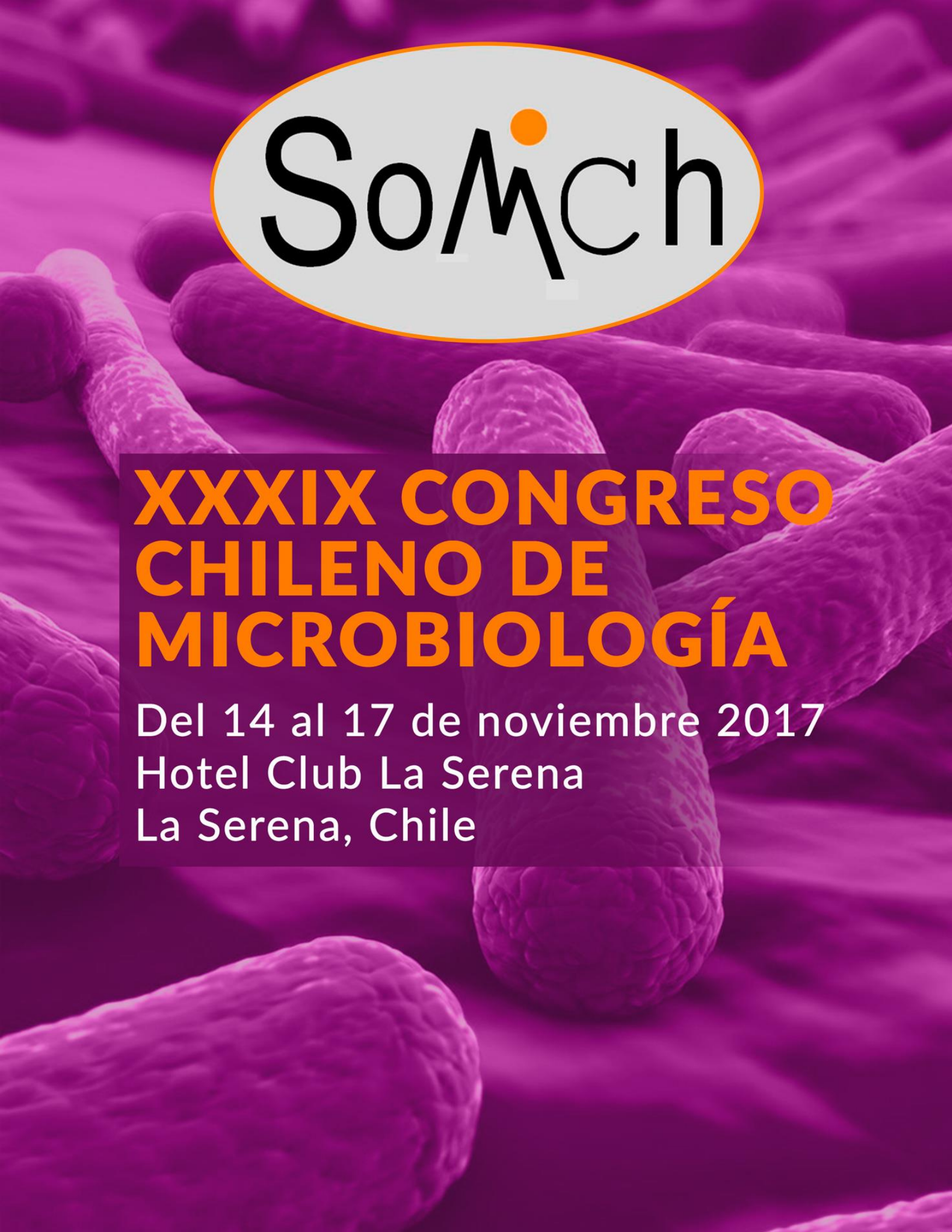

XXXIX CONGRESO CHILENO DE MICROBIOLOGÍA

Del 14 al 17 de noviembre 2017
Hotel Club La Serena
La Serena, Chile

XXXIX CONGRESO CHILENO DE MICROBIOLOGIA
XXXIX MEETING OF CHILEAN SOCIETY FOR MICROBIOLOGY

La Serena, November 14-17, 2017

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LECTURES

Global regulatory mechanisms in bacterial adaptation to sublethal levels of antibiotics

Sclavi B[1]. [1]LBPA, UMR 8113, CNRS, ENS Paris-Saclay.

Bacteria often encounter sub-lethal levels of antibiotics produced by other microorganisms in their environment. Furthermore, in a clinical setting, sub-lethal level of antibiotics can result from the incomplete assumption of the prescribed amounts or from concentration gradients that can exist in heterogeneous environments *in vivo*. Bacterial adaptation to the inhibition of the essential cellular processes targeted by these molecules allow the strain to survive long enough until the inhibitor is no longer present or in some cases to become resistant to the antibiotic via the emergence of novel mutations. However, the mechanistic details of these stress response pathways often remain to be described. In bacterial cells, inhibition of ribosome activity leads to induction of expression of ribosomal proteins and rRNA via the change in concentration of a secondary messenger molecule, (p)ppGpp, acting directly on the stability of the transcription initiation complex at ribosomal promoters. While the mechanism of transcription regulation by (p)ppGpp is a well-characterized effect, it remains to be established whether it suffices to explain the observed decrease in concentration of non-ribosomal gene products in these growth conditions. We measured the activity of two promoters that are differentially regulated by (p)ppGpp in the presence of sub-lethal concentrations of the ribosome-targeting antibiotic chloramphenicol. We found that the changes in the expression rate of the GFP reporter protein follows a similar pattern, independently of promoter sequence, resulting in both cases in an increase in GFP concentration. The comparison of the results obtained with two different reporter proteins, GFP and β-galactosidase, indicates that gene length plays a significant role in determining the rate of gene expression in the presence of ribosome inhibition. A quantitative model based on the decrease in translation and transcription processivity by chloramphenicol can explain these observations. These findings point to an important role of both transcription elongation and translation in the physiological feedback in response to inhibition of ribosome activity by naturally occurring antibiotics.

Dr. Sclavi obtained her undergraduate degree from Reed College in 1992 (Portland, OR, USA) and her PhD at Albert Einstein College of Medicine in 1998 (Bronx, NY, USA) with Michael Brenowitz and Mark Chance. Following her PhD, Dr Sclavi moved to Paris, at the laboratory of Henri Bucat in the Pasteur Institute, where she applied time-resolved footprinting techniques to the study of bacterial transcription complexes and notably to the elucidation of the different kinetic and structural intermediates in the pathways to promoter recognition and DNA melting by RNA polymerase. In 2000, she moved to the Institute Goustave Roussy with the group of Malcolm Buckle, and two years later they moved to the Ecole Normale Superièure de Cachan (now ENS Paris-Saclay).

uBiome LECTURE

The human microbiome grows-up: Transitioning from basic research to clinical applications

Goddard A[1]. [1]Vice President of Research and Development at uBiome, Inc.

The human microbiota encompasses the communities of commensal, symbiotic, and pathogenic microbes (bacteria, archaea, viruses, and fungi) that colonize the human body. The growing insight into the human microbiome (the genes encoded by these microbes) is challenging, and changing, our perspective on many human conditions and driving the development of novel therapeutics and new diagnostic tests. The microbiome is increasingly understood as a driver or modifier of disease and, thus, also as a target for intervention in these conditions. As a result, we are seeing a rapid evolution of approaches to leveraging the microbiome, with initial focus on the bacterial components, especially those in the large intestine. Drug and test development is moving to include the microbiome through new antibiotic strategies; bacteria as therapeutics; the microbiome as a diagnostic tool (through ribosomal 16S sequencing or metabolomics) for the presence of, or susceptibility to, disease; the microbiome as a predictive tool for selection of optimal therapy; and microbiota as a source of new therapeutic molecular entities. Specific examples include development of fecal microbiome transplantation or synthetically-derived microbiome therapeutics for treatment of *Clostridium difficile* infection, the SmartGut™ test (offered by uBiome) that sequences the V4 region of the bacterial 16S ribosomal RNA gene to measure microbial composition (in a CLIA-certified and CAP-accredited lab) to report microbial patterns that have been linked to conditions, and the observation that the intestinal microbiome influences the efficacy of immune checkpoint targeting agents in oncology.

Dr. Goddard is Vice President of Research and Development at uBiome, Inc. The inventor of over 500 patents in her previous roles, Dr. Goddard specialized in the integration of drug and diagnostic research and development, first at Genentech and then with Genomic Health. Dr. Goddard earned her Ph.D. in cancer genetics at the University of Toronto, then was a Postdoctoral Fellow at London's Imperial Cancer Research Fund. She spent thirteen years at Genentech, starting by leading high-throughput DNA sequencing for research and new drug discovery, then applying genomics and genomic technologies to companion diagnostics for oncology drug development.

ASM LECTURE

Non-canonical activation of OmpR drives acid and osmotic stress responses in single bacterial cells

Kenney LJ[1,2,3]. [1]Departments of Microbiology & Immunology and Bioengineering University of Illinois-Chicago. [2]Jesse Brown Veterans Administration Medical Center Jesse Brown Veterans Administration Medical Center. [3]Department of Biochemistry, Mechanobiology Institute, National University of Singapore.

Unlike eukaryotes, bacteria undergo large changes in osmolality and cytoplasmic pH. It has been described that during acid stress, bacteria internal pH promptly acidifies, followed by recovery. Using pH imaging in single living cells, we show that following acid stress, bacteria maintain an acidic cytoplasm and the osmotic stress transcription factor OmpR is required for acidification. The activation of this response is non-canonical, involving a regulatory mechanism requiring the OmpR cognate kinase EnvZ, but not OmpR phosphorylation. Single cell analysis further identifies an intracellular pH threshold ~6.5. Acid stress reduces the internal pH below this threshold, increasing OmpR dimerization and DNA binding. During osmotic stress, the internal pH is above the threshold, triggering distinct OmpR-related pathways. Preventing intracellular acidification of *Salmonella* renders it avirulent, suggesting that acid stress pathways represent a potential therapeutic target. These results further emphasize the advantages of single cell analysis over studies of population averages.

Supported by NIH 123640, VA IOBX-000372 and an RCE in Mechanobiology.

Dr. Kenney is a Professor of Microbiology at the University of Illinois-Chicago. Her laboratory studies two-component systems in bacteria that control gene expression at a single cell and nanometer level. The Kenney`s lab research is interested in signal transduction and the regulation of gene expression in prokaryotes. In particular, we are studying the two-component regulatory system EnvZ/OmpR that regulates the expression of outer membrane proteins as well as many other genes. Our present work focuses on how OmpR activates genes required for systemic infection (located on Salmonella pathogenicity island 2) in Salmonella enteric and recently, she is focus in exploring the bacterial molecular switch between virulence or dormancy, Salmonella Lifestyle Choices.

GrupoBios LECTURE

Microbial analysis using quantitative microscopy

Montero C[1]. [1]Biotek GrupoBios.

In this presentation we will demonstrate the application of quantitative microscopy to the analysis of a number of microbial models common to bacteriology, virology, mycology, and parasitology. The quantitative microscopy tool box consists of an automated digital microscope, an array of stains and fluorescent probes, and s/w for multi-parameter processing and analysis of acquired images resulting in both qualitative and quantitative data outcomes. Applications using live and fixed microorganisms will be highlighted via individual case studies, including quantitating plaque assay viral titers in 96-well plates using montage and stitching; calculating bio volume and morphology characteristics of bacterial and fungal matrices using z-stacking; and monitoring effects of phagocytic inhibition on uptake of bacteria and yeast.

LECTURE

Proyecto de ley que crea el Ministerio de Ciencia y Tecnología

Hamuy M[1]. [1]Presidente de la Comisión Nacional de Investigación Científica y Tecnológica CONICYT

Dr. Mario Hamuy es astrónomo chileno, profesor de Astronomía de la Universidad de Chile y del Observatorio Cerro Calán. Es reconocido por su trabajo de observación de diferentes clases de supernovas, especialmente las supernovas de Tipo Ia y Tipo II como medidas de distancia cósmica. En agosto de 2015 recibió el Premio Nacional de Ciencias Exactas de Chile. Fue Director del Instituto Milenio de Astrofísica (MAS), donde hoy se desempeña como Investigador Senior. Actualmente es Presidente de la Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) y Asesor Científico de la Presidencia.

LECTURE

Impact of synonymous codons (silent mutations) in the expression, folding and function of proteins

Moreira S[3], Arias L[3], González D[3], Avalos F[1], Tello M[1], Levicán G[2], **Orellana O[3]**. [1]Centro de Biotecnología Acuícola, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. [2]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. [3]Programa de Biología celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile.

Historically, the genetic code have been considered as redundant since most amino acids are encoded by more than one (2, 4 or 6) codon. Thus, mutations replacing synonymous codons are called as silent since no amino acid change takes place. However, the existence of synonymous codon bias at intra and inter genomic levels has been described. Therefore, the question as to whether synonymous codons are effectively redundant or have specific roles that explain codon bias has emerged. Recent studies have shown that changes in synonymous codons may alter the cellular proteome. Different hypothesis have been proposed to explain these effects as changes in the structure, stability and translation efficiency of mRNAs or effects on protein folding. Levels of aminoacyl-tRNAs that decode synonymous codons have been considered as potential effectors altering translation efficiency of synonymous codons. Our laboratory has approached some of these questions through the prediction of the effect of synonymous codons on the interaction of regulatory sRNAs and target mRNAs in bacteria. Also the folding and function of Hfq (RNA chaperone) from bacteria and PGK-1 (phosphoglycerate kinase-1) from yeast have been studied. The effect of changes in tRNA concentration on translation and folding of proteins that participate in the cell cycle have been studied in yeast. Results suggest that both the level and folding of proteins are altered by synonymous mutations. Furthermore, changes in synonymous codons as well as tRNA levels alter growth and morphology of either bacteria or yeast. Supported by FONDECYT Regular 1150834 (OO) And 1170799 (GL), FONDECYT Postdoctorado 3150366 (SM), Fellowship CONICYT Magister Nacional 22151224 (LA) and Grant USA 1555 (MT).

Dr. Orellana se formó a nivel de pregrado en la Universidad de Concepción y doctorado en la Universidad de Chile, ambas bajo la dirección del Dr. Jorge Allende y de postdoctorado en Yale University, Estados Unidos con supervisión del Dr. Dieter Soll. Desde este período, consolidó una línea de investigación en el área de la relación entre la estructura molecular y la función biológica de los ácidos ribonucleicos (tRNA, sRNA) y las proteínas involucradas. Organizó en Chile junto al Dr. M. Ibba y colaboradores nacionales el 22th International tRNA Conference el año 2012. Ha sido Presidente de la Comisión Académica del programa de Doctorado en Ciencias Biomédicas, Director del Programa de Biología Celular y Molecular y Subdirector de Investigación del ICBM y Director de Investigación de la Facultad de Medicina de la Universidad de Chile. Ha sido miembro de comités de Fondecyt, Becas Chile, Comisión Nacional de Acreditación, Sociedad de Bioquímica de Chile, Sociedad de Microbiología de Chile (presidente entre 2009 y 2013).

LECTURE

Engineering lactococci and lactobacilli for human health

Bermúdez-Humarán LG[1]. [1]Instituto Micalis INRA-Francia.

Food-grade lactic acid bacteria (LAB) are good candidates for the development of oral vectors, and are attractive alternatives to attenuated pathogens, for mucosal delivery strategies. In this presentation, I will summarize our recent results on the use of LAB as mucosal delivery vectors for therapeutic proteins. Most of this work has been based on the model LAB, *Lactococcus lactis*, which is suitable for the heterologous expression of therapeutic proteins. Recombinant lactococci and lactobacilli strains expressing either anti-inflammatory cytokines, antiproteases or antioxidant enzymes have been tested successfully for their prophylactic and therapeutic effects in different murine models of colitis. We believe that these various coherent findings demonstrate the potential value of using LAB, particularly lactococci and lactobacilli strains, to develop novel vectors for the therapeutic delivery of proteins to mucosal surfaces. Further tests and in particular human clinical trials are now important next steps to conclude on the benefit of these approaches for human health.

Dr. L. Bermúdez is a senior researcher at Micalis institute which is a "joint research unit" associating INRA (French National Institute for Agricultural Research) and AgroParisTech with the aims of developing innovative research in the field of Food and Gut Microbiology for human Health. His research is mainly focused on the therapeutic uses of recombinant lactic acid bacteria (LAB) and probiotics to modulate the host immune response at mucosal surfaces. He is among the first world leaders in the field of use of these live vectors for the development of new mucosal vaccines. He has a strong expertise on Microbiology, Molecular Biology, Vaccinology and Animal models. He has also succeeded to obtain grants from the French Ministry of Research, the French Cancer Research Association (ARC) and the European FP6 project: Pathogen Combat, and he has been recognized with several awards. He has published 92 articles in journals with international peer review and has 6 patents.

Closing LECTURE

Los Beatles y El Universo

Gomberoff A[1]. [1]Facultad de Ingeniería y Ciencias de la Universidad Adolfo Ibáñez

Science is everywhere. This was suggested by Michael Faraday in his legendary series of conferences for all audiences "The chemical history of a candle", where he warned "There is nothing better, there is no more open door through which you can enter to study natural philosophy, that considering the Physical phenomenon of a candle ". Maybe today a candle does not have the impact it generated in the youth of 1848. Maybe it's the music, and why not, rock and roll, a much wider and colorful door to enter the study of physics.
Starting with the audition of three songs from the 1966 Beatles classic "Revolver" album, we will introduce ourselves to several scientific topics. These are relevant both to our understanding of the universe and to making this masterpiece of contemporary music possible. The electric guitar and the electromagnetic spectrum, thermodynamics and synthetic sounds, the expansion of the universe and the sound of the Hammond organ will be some of the topics that we will address in a talk aimed at all audiences.

Dr. Gomberoff es licenciado y doctor en física de la Universidad de Chile y postdoctorado en el Centro de Estudios Científicos de Valdivia (CECS) y en la Universidad de Syracuse, EE.UU. Actualmente, académico de la Facultad de Ingeniería y Ciencias de la Universidad Adolfo Ibáñez. En gestión y administración universitaria, fue creador y primer director de la carrera de Ingeniería Física en la UNAB, y posteriormente Vicerrector de Investigación y Doctorado de la misma Universidad. Como divulgador de la ciencia, colabora con la revista Qué Pasa escribiendo artículos quincenales, los que se han recopilado en tres libros. Su trabajo de divulgación ha sido premiado tanto en Chile como en España.

SYMPOSIA

S01

Molecular mechanisms of microbial pathogenesis. Implications in the development of vaccines

Chair: Dr. Alexis Kalergis

Understanding the mechanisms used by *Salmonella enterica* serovar Typhimurium to hijack the immune system and cause systemic diseases

Bueno SM[1]. [1]School of Biological Sciences, Pontificia Universidad Católica de Chile. Associate Researcher, Millennium Institute on Immunology and Immunotherapy.

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is an important gastrointestinal pathogen. *S.* Typhimurium has several virulence genes encoding virulence factors to colonize the epithelium and to survive inside phagocytic cells. We have demonstrated that production of IL-10 during *S.* Typhimurium infection is a key determinant to cause systemic dissemination in mice. After 5 days of oral infection, C57BL/6 mice show elevated levels of IL-10 in serum, with no increase in the levels of pro-inflammatory cytokines, despite a high bacterial load in all the tissues evaluated. Accordingly, high levels of IL-10 mRNA are detected in ileum, spleen, and liver; and these tissues are infiltrated with diverse IL-10 producing cells, such as neutrophils, T cells, B cells, dendritic cells and macrophages. Moreover, FOXP3+ regulatory T cells are found in the spleen of infected mice. The relevance of IL-10 in the infective cycle of *S.* Typhimurium is supported by the fact that mice unable to produce IL-10 (IL-10-/-) exhibit an increased inflammatory response, less bacterial load in spleen and liver and less mortality, as compared to IL-10 producing mice. We observed that adoptive transfer of IL-10 producing B or T cells to IL-10-/- recipient mice restores the susceptibility observed in WT mice, indicating an important role of these cells during infection. These results demonstrate that *S.* Typhimurium use virulence mechanisms to induce the production of IL-10 by the induction of regulatory cells in the infected host.

The critical role of TSLP in the pathogenesis caused by human Metapneumovirus; Norovirus-host cell interactions; and a projection towards the production of vaccines manufactured in Chile

Lay MK[1,2], Céspedes PF[1], Palavecino CE[1], León MA[1], Díaz RA[1], Salazar FJ[1], Méndez GP[3], Bueno SM[1,4], Kalergis AM[1,4,5], Parola A[6], Willans N[7], Flores-Gorigoitía C[2], Escobar D[2], Ardiles P[2], Cuellar, S[2], Campillay C[2], Carvajal J[2], Valdivia I[2], Estes MK[8]. [1]Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile. [3]Departamento de Anatomía Patológica, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [4]INSERM U1064, Nantes, France. [5]Departamento de Reumatología,

Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [6]Fundación Pablo Cassará, Instituto de Ciencia y Tecnología Dr. Cesar Milstein, Buenos Aires, Argentina. [7]Departamento de Ingeniería Comercial de la Universidad de Antofagasta, Antofagasta, Chile. [8]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA

Human metapneumovirus (hMPV) is a leading cause of acute respiratory tract infections in children and the elderly. The mechanism by which this virus triggers an inflammatory response remains unknown. Here we evaluated whether the thymic stromal lymphopoietin (TSLP) pathway contributes to lung inflammation upon hMPV infection. We found that mice lacking the TSLP receptor deficient mice ($tslpr^{-/-}$) showed reduced lung inflammation and hMPV replication. These mice displayed a decreased number of neutrophils as well a reduction in levels of TARC/CCL17, IL-5, IL-13, and TNF-α in the airways upon hMPV infection. Further, blockage of TSLP or OX40L with specific Abs reduced lung inflammation and viral replication following hMPV challenge. These results suggest that activation of the TSLP pathway is pivotal in the development of pulmonary pathology and hMPV replication. In addition, human noroviruses (HuNoVs), including Norwalk virus (NV), are the cause of more than 95% of non-bacterial epidemic gastroenteritis worldwide. It is not clear whether HuNoV infections induce interferons (IFNs). In this study, we tested whether the non-neoplastic human hepatocyte cell line, PH5CH8, allows NV replication and whether it is enhanced after the blocking of the type I IFN pathway. We found that NV stimulates the type I IFN response in PH5CH8 cells and the blocking of this response by using a cocktail anti-IFN Ab, enhanced NV protein expression in these cells. These results suggest PH5CH8 cells may be a useful *in vitro* model for establishing a permissive cell culture system for HuNoV. Besides, currently, all vaccines in Chile are manufactured abroad, which generates a high dependence on global contingency and generates a high expenditure from the national budget. In the case of a viral pandemic, our population could be at a high risk of susceptibility, since we do not have a vaccine manufacturing plant in the country to be able to produce vaccines that could save many lives. That is why Chile urgently needs a laboratory and a manufacturing plant to develop vaccines that are required by our population. The creation of the "Atacama Desert Vaccine Laboratory" could aid in improving the health of the Chilean population.

New strategy for the development of a vaccine against Shigatoxin-producing *Escherichia coli* (STEC)

Montero D[1], Del Canto F[1], Oñate A[1], Salazar JC[1], **Vidal R[1].** [1]Laboratorio de *Escherichia coli* Patógenas (LEP), Programa de Microbiología y Micología, Facultad de Medicina, Universidad de Chile.

Shiga-toxin producing *Escherichia coli* (STEC) is causative agent of acute diarrhea, dysentery and hemolytic uremic syndrome (HUS), a life threatening clinical picture that can cause death or lifelong impairments. In Chile, STEC infections are endemic and they account for 90% of the HUS cases. Currently, there is no treatment for STEC infections. Antibiotics are not recommended because they would increase secretion of the shiga-toxin, the main STEC virulence factor involved in the development of HUS. In addition,

there is no effective vaccine available to prevent STEC infection in humans. Therefore, it is necessary to look for new treatment alternatives or to develop vaccines to protect humans against this pathogen. Recently, in our research group, we found that three STEC proteins are recognized *in vivo* during the infection and stimulate IgG and IgA specific antibodies. Since these proteins are produced by the most epidemiologically relevant STEC serogroups, they could be suitable candidates to develop vaccines. The main goal of our work is to develop chimeric proteins based on immunoreactive epitopes of these three antigens as potential protective immunogens against STEC infections. Immunoreactive peptides (epitopes) were identified for each protein by peptide array using sera from patients with diagnosis of HUS. These peptides were then synthesized and prove their antigenic properties and select the most immunoreactive epitopes using the same sera from patients who suffered HUS. Currently we are working on design chimeric proteins using at least one epitope from each protein and modeling the tertiary structure *in silico* to corroborate the proper exposure of the immunogenic peptides. The next step will be synthesize the chimeras and demonstrate their reactivity to epitope-specific antibodies and HUS-sera.

Interference with the immunological and neurological synapses as virulence mechanisms of RSV. Implications for vaccine design

Kalergis AM[1]. [1]Millennium Institute on Immunology and Immunotherapy. Facultad de Medicina. Pontificia Universidad Católica de Chile. Director

Human Respiratory Syncytial Virus (hRSV) is the leading cause of bronchiolitis and pneumonia in young children worldwide. The recurrent hRSV outbreaks and reinfections are the cause of significant public health burden and associate with an inefficient viral immunity, even after disease resolution. Furthermore, hRSV can also cause severe symptoms, such as dyspnea and chest wall retractions. Recently, neurological symptoms have also been associated with hRSV-respiratory infection and include seizures, central apnea, lethargy, feeding or swallowing difficulties, abnormalities in muscle tone, strabismus, abnormalities in the CSF and encephalopathy. Although several mouse- and human cell-based studies have shown that hRSV infection prevents naïve T cell activation by antigen-presenting cells, the mechanism underlying such inhibition remains unknown. We have shown that the hRSV nucleoprotein (N) could be at least partially responsible of inhibiting T cell activation during infection by this virus. Early after infection, the N protein is expressed on the surface of epithelial and dendritic cells, after interacting with trans-Golgi and lysosomal compartments. Further, experiments on supported lipid bilayers loaded with peptide-MHC complexes, showed that surface-anchored N protein prevented immunological synapse assembly by naive CD4+ T cells. Synapse assembly inhibition was in part due to reduced TCR signaling and pMHC clustering at the T cell-bilayer interface, suggesting that protein N interferes with pMHC-TCR interactions. Moreover, N protein co-localized with the TCR independently of pMHC, consistent with a possible interaction with TCR complex components. Based on these data, we conclude that hRSV N protein expression at the cell surface of infected cells inhibits T cell activation. These data suggest that inhibition of synapse assembly can be a major virulence factor that contributes to impairing acquired immunity and enhances

susceptibility to reinfection by hRSV. These data allowed the generation of an RSV vaccine, which showed protection in several animal models and is currently under evaluation in human clinical trials.

S02

Molecular biology applied to clinical diagnosis

Chairs: Dr. Claudia Saavedra and Dr. Mauricio Farfán

Impact of molecular diagnostic in clinical microbiology

Tapia C[1]. [1]Programa de Microbiología y Micología, ICBM, Facultad de Medicina, Universidad de Chile.

The diagnosis of infectious diseases has been highly improved by the development of molecular techniques based on the polymerase chain reaction (PCR) in the clinical laboratory. The high sensitivity of these techniques has allowed the detection of viral, bacterial and fungal genetic sequences in clinical samples, allowing the patient to be diagnosed early and initiate a timely therapy. Its high specificity has allowed to constitute the best alternative for the diagnosis of viral, bacterial and fungal pathogens, replacing or complementing traditional microbiology (eg: diagnosis of viral encephalitis, respiratory and gastrointestinal tract, among others). At present, the trend is to use platforms that detect multiple pathogens, in less time and in an automated way, also allowing the transmission of the results to the hospital network and even to the cell phones of the treating physicians. Currently, these methodologies have been widely used in clinical laboratories, with numerous kits and equipment being available for *in vitro* diagnostic use (IVD). In this way, these molecular techniques have gone from being used only in research and in highly specialized laboratories, to routine diagnostic laboratories.

Molecular point of care testing

Farfán M[1]. [1]Pediatría, Facultad de Medicina, Universidad de Chile.

According to the College of American Pathologists Point of care testing (POCT) are tests designed to be used at or near the site where the patient is located that do not require permanent dedicated space, and are performed outside the physical facilities of the clinical laboratories. In health care facilities POCT has been mainly limited to antigen-based tests, because these techniques have a short turnaround (30-60 min), low cost and no trained-personnel are required. However, these tests have insufficient sensitivity, and randomized controlled trials have failed to show clinical or health economic benefits. On the other hand, Polymerase chain reaction (PCR)-based test are highly accurate, but turnaround is generally 24–48 h, requires specialist, centralized laboratory facilities and are expensive. Considering the above, in the last decade several rapid molecular testing platforms for pathogens have been developed with broadly equivalent diagnostic accuracy to laboratory PCR, and some of these platforms are potentially deployable as POCT. In this talk, we will discuss the molecular POCT test platforms available in terms of sensitivity, feasibility, cost and the impact of health. There are growing evidence that molecular POCT leads to improvements in the rate of pathogen detection, improved antiviral use, rationalization of hospital isolation facilities and reduction in length of stay.

However, there are several consideration and diagnostic algorithms that are necessary to develop in order to implement routine molecular POCT as part of standard care.

Diagnosis and clinical monitoring of HIV/AIDS

Acevedo W[1]. [1]Infectología Hospital Dr. Lucio Cordova, Santiago, Chile.

The last report about HIV/AIDS worldwide of WHO and UNAIDS shows that Chile has the worst results in Latin America about screening and prevention of the disease. For this reason the Ministry of Health had raised new policies about public campaigns involving the importance of the test for HIV in the population but in special among the youth who were the most affected in the last decade. The 90-90-90 goals for HIV/AIDS needs to be reached by 2020, for this reason, the importance of the diagnosis, access to treatment and undetectability of patients and therefore the importance of new techniques from ELISAs to Molecular Biology in the Diagnosis, Monitoring and Control of the infection is vital to reach the goals. Techniques as PCR qualitative, PCR quantitative (Viral Load) and sequencing looking for drug resistance mutations in the HIV genome has become the standard of care for this pathology. In this presentation we will show them and discuss about the importance in the patients management. The qualitative PCR is used, as the main technique for the confirmation of newborn babies due to is not possible using antibody detections, which belong to the mother, in the prevention of vertical transmission of the virus. The quantitative PCR (Viral Load) is one of the most important assays in the routine to control the patients, because can show how is the virus responding to treatment. A total control of the virus replication by the drugs can be measured using this technique. The genotyping of HIV or Genetic resistance test of HIV can determine mutations in different genes of HIV. Those mutations are responsible for the resistance of the virus to the drugs. Minimum to maximum complexity in the routine clinical laboratory.

S03

Engineering microbes driven by systems and synthetic biology for biotechnological applications

Chair: Dr. Ignacio Poblete

Engineering *Pseudomonas putida* driven by systems and synthetic biotechnology for the synthesis of biopolymers

Poblete-Castro I[1]. [1]Biosystems Engineering Laboratory, Ciencias Biológicas, Universidad Andrés Bello, Chile.

One of the most sustainable systems for producing chemicals at industrial scale is through microbial fermentation of various feedstocks. This has yielded a large portfolio of biochemical compounds including amino acids, fuels, proteins, and biopolymers. Bacterial strains from the genus *Pseudomonas* have been employed as cell factories for the synthesis of Polyhydroxyalkanoates (PHAs), a sort of biopolymer with similar mechanical a physical properties as compared to conventional plastics, which is one the most promising polymers to replace oil-based plastics in the near future. Despite the advances, one of the main drawbacks of PHAs for better market positioning is the high cost associated to the carbon source and downstream processing for product recovery. Driven by *in silico* pathway design and metabolic engineering we have enhanced the production of medium-chain-length PHAs in *P. putida* H using benzoate as the unique carbon source, elevating the biopolymer synthesis by more than 80% in batch cultures. Moreover, we have recently programmed *P. putida* with a novel genetic circuit, which enabled the autolysis of the cell more than 95% of cell mortality at any desired point of the fermentation, avoiding expensive extraction methods for the recovery of PHAs. We have demonstrated that the combination of systems and synthetic biology approaches to engineering biological systems is one of the most powerful tools to fully exploit the production capacity of tailor-made PHAs in metabolically engineered *Pseudomonas putida* strains on low-cost substrates.

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Open source biotechnology

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Inspired by the impact of Free/Libre and Open Source Software (FLOSS), an open source ethos is gaining traction among hardware developers and users. This approach is not only becoming ubiquitous in DIY movements, maker spaces, high schools, biohacking communities and art collectives but also being embraced by the scientific community. Hundreds of labs around the globe design and self-manufacture their own equipment, provide open access to their research, document projects online, share blueprints and grant open access to their inventions. For instance, the advent of easy-to-use open microcontrollers, off-the-shelf electronics and customizable manufacturing technologies such as 3D printers and laser-cutting machines, has enabled a diverse community of

open labware developers, users and tinkerers. The use of online platforms for project documentation and sharing, along with the implementation of collaborative practices borrowed from the FLOSS community, is giving rise to crowdsourced development of advance instrumentation. These modes of development are lubricated by licenses that guarantee freedom to access, study, use, modify and re-distribute technology source code, design files and derived works (for profit or non-profit). Here, I will address efforts to promote this approach in biological engineering. I will describe i) the integration of low-cost and open-source hardware, software and genetic resources for imaging microbiological phenomena in scales ranging from single colonies to entire plates, ii) IP-free DNA fabrication tools, iii) open source legal framework to facilitate material transfer and sharing between researchers and iv) international movements that promote the adoption of these frameworks with the aim of achieving more inclusive, efficient, affordable and equitable ways of producing technology, innovation and knowledge in general.

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Bacterial stimulation of *Arabidopsis thaliana* growth and stress tolerance by volatile signals from *Paraburkholderia phytofirmans*: key metabolites and their production

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Plant growth promoting rhizobacteria (PGPR) have been shown to improve plant growth and tolerance to environmental stress under different experimental systems, involving the interaction of a single bacterial strain with a plant host. However, the bacterial mechanisms underlying these beneficial effects are still a matter of intensive research for most of the plant-bacterium associations described to date. *Paraburkholderia phytofirmans* PsJN can stimulate growth and tolerance to salinity and drought in a variety of plant hosts, including *Arabidopsis thaliana*. In this work, we have analyzed the role of different growth promotion traits on PsJN-mediated effects by recombinational mutagenesis of bacterial genes, and have explored the role of bacterial volatile emissions on plant growth and stress tolerance. The results obtained indicate that plant growth promotion by *P. phytofirmans* is mainly achieved through volatile-mediated signalling and that direct contact with the plants is not required to produce a physiological response that enhances growth rate, yield and stress tolerance in general. Furthermore, these effects were shown to influence the complete life cycle of the plants, even when the volatile stimulus is applied only at the early stages of development. Active volatile signals were identified by GC-MS and found to include the compounds 2-undecanone, 3-methyl butanol and 1-heptanol, which can partially mimic the effects of PsJN on *A. thaliana* when added to the plants in the absence of bacteria. In summary, these results describe an efficient phytostimulation system, previously uncharacterized for the genus *(Para)burkholderia*, that uses a discrete and novel set of active compounds. This finding

suggests that volatile-mediated plant growth stimulation is more widespread than previously thought, even within well described PGPR belonging to the phylum Proteobacteria, and highlights the relevance of this type of signalling in beneficial interactions in the rhizosphere ecosystem.

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S04

Fungal secondary metabolism: evolution, regulation and applications

Chair: Dr. Inmaculada Vaca

Biotechnological applications of secondary metabolites produced by filamentous fungi

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Fungi are among the most important groups of microorganism in modern biotechnology, especially for pharmaceutical and food industry applications. These organisms are producers of secondary metabolites with numerous biological activities such as antibiotics, antivirals, antimycotics, cytotoxics, immunosuppressives and pigments. The secondary metabolites are molecules, usually of low molecular weight, with complex and variable chemical structures. Even though during the last decades of the 20th century there was a decline in the search for fungal natural compounds with biological properties, currently there is a revival, led mainly by the search of new antibiotics to face bacterial resistance to available antibiotics. In this lecture, I will show our advances in the search of secondary metabolites from marine and Antarctic filamentous fungi, and their potential applications in several fields. In particular, I will show three examples: i) fungal secondary metabolites that could be used as novel pigments; ii) fungal secondary metabolites with antibiotics properties; iii) fungal secondary metabolites that would be useful for Alzheimer's disease. These examples reflect that fungi remain as promising producers of compounds useful to mankind.

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Secondary-metabolite gene clusters in filamentous fungi: an evolutionary overview

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Filamentous fungi have a great ability to synthesize secondary metabolites. Secondary metabolites are chemical compounds that although are not fundamental to the fungal survival, they have several ecological roles, such as chemical communication, chemical defense, and as virulence factors for plants and animals. From the human point of view, fungal secondary metabolites have been widely studied due to their biotechnological importance. However, from the perspective of the fungal biology, secondary metabolites have been less studied. In filamentous fungi, secondary metabolites are produced by biosynthetic enzymes that are usually encoded by physically clustered genes, forming genomic structures known as secondary-metabolite gene clusters. The current availability of genome sequences has revealed the huge biosynthetic potential of filamentous fungi, and has opened new opportunities for the study of secondary-metabolite gene clusters from the evolutionary perspective. In this context, I will describe the main evolutionary processes driving the formation of secondary-metabolite gene clusters. Special emphasis will be placed on the fungal speciation processes, their

relationship with the production of extracellular secondary metabolites in a species specific manner, and how the identification of these metabolites can help to resolve taxonomic discrepancies or to define the formal description of novel species. To addressing these issues, several study cases in different filamentous fungi will be shown.

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AP-1 transcription factors regulate the biosynthesis of secondary metabolites in response to oxidative stress in fungi

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Reactive oxygen species (ROS) trigger and modulate different morphogenic processes in filamentous fungi, and have been shown to participate in the regulation of the biosynthesis of some secondary metabolites. Some bZIP transcription factors, such as Yap1, AtfA and AtfB, have been shown to mediate resistance to oxidative stress and to have a direct or indirect role in the regulation of secondary metabolism. In this work we describe that in *Penicillium chrysogenum*, penicillin biosynthesis is stimulated by ROS, generated by the addition of H_2O_2 to the culture medium, and that this regulation is mediated by the transcription factors PcYap1 and RsmA. Attenuation of expression of both proteins by RNAi resulted in similar phenotypes, characterized by increased levels of ROS in the cell, reduced conidiation, higher sensitivity of conidia to H_2O_2 and a decrease in penicillin production. PcYap1 binds in vitro to a previously identified regulatory sequence in the promoter of the penicillin gene *pcbAB*: TTAGTAA, whereas RsmA binds to the sequence TGAGACA, located 67 bp upstream from the PcYap1-binding site. PcYap1 plays in *P. chrysogenum* a similar role in the oxidative stress response to that described in other filamentous fungi for Yap1 homologs; however, its role as direct activator of transcription of a secondary metabolite gene had not been previously reported. RsmA is an important mediator in the oxidative stress response of *P. chrysogenum*, a function that is not conserved in all orthologs from other filamentous fungi studied so far.

S05

Microbial amyloid: a problem for some - a solution for others

Chair: Dr. Rosalba Lagos

Turning ploughs into swords: Exploring the cutting edge between function and disease in a bacterial intracellular amyloid

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Bacteria have functional extracellular amyloids that are used, e.g., to scaffold biofilms. However, no natural amyloid 'proteinopathy', i.e. amyloids toxic or detrimental to bacterial proliferation as those triggering human neurodegenerative diseases, had been found yet. RepA protein initiates DNA replication of the *Pseudomonas* plasmid pPS10, having close relatives among many plasmids in Gram-negative bacteria. Its N-terminal winged-helix domain (WH1) acts as dimerization interface, enabling RepA to repress its own transcription. On the contrary, RepA monomers are the species active in DNA replication and, once this is completed, remain bound to DNA to establish trans-acting nucleoprotein ('handcuffed') complexes that inhibit premature replication rounds. We have recently discovered that such complexes are built on an amyloid oligomer made by the WH1 domain, thus being the first intracellular functional amyloid. We have engineered the WH1 domain of RepA to assemble as amyloid fibres upon binding to short specific plasmid DNA sequences. RepA-WH1 amyloid fibres are bundles made of intertwined tubular protofilaments, which are made of structurally distorted protein monomers. RepA-WH1 causes in *E. coli* an amyloid proteinopathy, which is *vertically* transmissible from mother to daughter cells, templating its conformation by cross-seeding *in vitro* and *in vivo*. However, RepA-WH1 is not infectious, i.e. is a 'prionoid'. Bacterial lineages maintain two mutually exclusive strains of RepA-WH1 amyloids: either multiple globular particles that inhibit cell division, or a single elongated aggregate, mildly detrimental to growth. The bacterial Hsp70 chaperone DnaK modulates the phase transition between both amyloid strain. Amyloidogenic oligomeric precursors of RepA-WH1 locate at the bacterial nucleoid. Concerning its mechanisms of cytotoxicity, RepA-WH1 builds pores in lipid vesicles analogous to the *E. coli* inner membrane, leading to leakage of their contents. Systems Biology approaches show that RepA-WH1 amyloidosis in bacteria also implies the generation of ROS and the co-aggregation with the protein of essential cellular factors involved in the response to oxidative stress. This scenario of three concurrent cytotoxic events has counterparts in human amyloidoses. RepA-WH1 provides a unique window to survey the minimal landscape of an amyloid proteinopathy, endorsing this prion-like protein as a generic bacterial model for amyloid diseases.

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Functional amyloids in the microbial world: lessons from a bacterial toxin

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Amyloid fibrils are highly organized protein aggregates that have been classically associated to mammal's neurodegenerative pathologies such as Alzheimer's, Parkinson's, and Huntington's disease. However, several examples of amyloids playing a biological role have been described in many organisms: from bacteria and fungi, to insects, fish, and mammals. In bacteria, these "functional amyloids" can participate in biofilm formation, host cell adhesion and invasion, aerial hyphae growth, and plasmid replication. Moreover, it was proposed that the amyloid is a kind of ancestral folding that could be adopted by many proteins, drawing an scenario where multiple amyloids would coexist and interact inside the cell. However, very little is known regarding such possible amyloid-amyloid interactions, and regarding how the cells handle such structures. A remarkable example of a bacterial amyloid is the antimicrobial peptide microcin E492 (MccE492) produced by *Klebsiella pneumoniae*, which *Enterobacteriaceae*-targeted toxic activity is modulated by amyloid formation. In this talk, I will discuss our main findings regarding key biochemical aspects of MccE492 amyloidogenesis, its relation with a novel amyloid-forming microcin that is co-produced with it, and regarding possible ecological and physiological implications of microcin production and amyloid formation.

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Expanding the amyloid concept: catalytic amyloids and nanomaterials

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Amyloids are a class of highly ordered and structured aggregated polypeptides. These structures have been classically associated to pathological processes involved in different diseases such as Alzheimer's and Parkinson's disease. However, an increasing number of reports in recent years of novel amyloids involved in the normal physiology of organisms has forced to reconsider the role of amyloids in Biology. Moreover, the molecular details that have recently surfaced in the literature have exposed an unexpected complexity in these structures. Their regular architecture and structural coherence allows for a repetitive positioning of the constituent residues and their side chains, which can lead to the development of potentially active biosurfaces. Our group has recently developed *de novo* amyloids that present hydrolytic activity towards biologically relevant molecules such as adenosine triphosphate (ATP). These novel catalytic amyloids were rationally designed to mimic the active sites of enzymes involved in the biochemical processing of nucleotides. Our results show that reactive amyloids can emerge when certain combinations of residues are specifically arranged in their sequences. The development of catalytic amyloids can open up the door for the design of novel bio-nanomaterials that can combine the unique physical properties of amyloids with directed reactivity.

FONDECYT 11160554

S06

Human microbiology: History and future of the next biomedical revolution

Chairs: Dr. Claudia Saavedra - Dr. Juan Ugalde

A decade of studies on the human microbiome: challenges and promises

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Human microbiology has traditionally relied on culturing techniques to study pathogenic bacteria and viruses. However, recent developments in sequencing methods and bioinformatics analysis tools now enable culture-independent analysis of the complex microbial communities associated with the human body. In the past 10 years, human microbiome research has yielded many new insights in determining the composition and functionality of these microbial assemblies. Some of the most important conclusions are: 1) The human-associated microbial communities vary primarily between anatomical sites, but also per individual and over time. 2) The gut microbiome, the largest microbial community in the human body both in terms of absolute numbers as well as number of species, serves important functions such as breaking down dietary components that human enzymes cannot digest, synthesis of short chain fatty acids and other small molecules that serve as messengers throughout the human body, and shaping our immune system and anatomical structures. 3) The composition of the microbiome is shaped by a multitude of variables, including diet, lifestyle factors, medicine use, and health status. This opens the possibility for clinicians to use microbiome analysis as a tool to assess a patient's medical condition or treatment response.

Microbial diversity in urban environments: Interactions between humans and the built environment

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The study of the human microbiome has largely focused on the microorganisms that live within our body. However, in our daily life we encounter multiple microorganism in the environment that surrounds us, which could have positive or negative impacts in our microbial diversity, health, and general well-being. A large fraction of the world population inhabits urban environments, and only recently the distribution of microbes and factors that affect their diversity has started to be explored. In this presentation, I want to highlight some results from studies of urban microbiomes in different parts of the world, including preliminary results obtained from sampling performed at the Subway system in Santiago de Chile. This information will help us to comprehend the diversity of microbes that inhabits the urban environment, and possible interactions with the human microbiome.

From the microbiome back to the bench: studies in microbial interactions associated to periodontitis

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The growing interest in characterizing the microbiome associated with all kind of human diseases has produced a large amount of data that opens up new opportunities to investigate both the etiology and potential therapeutic strategies for these diseases. The oral cavity corresponds to an interesting niche that is receiving more and more attention due to the potential extra-oral effects of associated microbial species. Periodontitis correspond to an inflammatory condition that affects the supporting structures of teeth, resulting in the destruction of the connective tissue attachment, alveolar bone resorption, and eventual tooth loss. This disease is likely to be triggered by an imbalance between the host defenses and the microbial communities present in the subgingival sulcus, leading to shifts in the community structure of the subgingival microbiome. Such periodontitis-associated communities are more diverse and show higher biomass compared to health-associated communities. However, the exact mechanisms that lead to the establishment of these dysbiotic communities have not been fully determined. Therefore, seems to be necessary to re-visit all this data obtained from high-throughput studies and, by using more classical strategies, try to understand which inter-species mechanisms lead to the transition from health to disease.

S07

Microbial ecology of extreme systems

Chair: Dr. Raquel Quatrini

Acidophilic microbial communities from the Copahue Geothermal System. Rio Agrio as an environmental gradient model system

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The geothermal system Copahue–Caviahue is located, in the Argentinian province of Neuquen. The Copahue Volcano is a stratovolcano with an acidic crater lake (pH 0.2–1.1) that feeds four hot springs spanning both Argentinian and Chilean territory. They exhibit wide ranges of pH (1-7) and temperature (20-90°C) and are colonized by diverse extremophilic life forms. The main water drainage in the system is Rio Agrio, which starts as a hot spring (pH 0.3–2.3) and gets cooled and diluted with tributary inflows farther downstream. This river discharges its waters in the Lake Caviahue (pH 2.1–3.7) also acidic. The overflows waters give origin to the Lower Rio Agrio (pH 2.1–6.0) which flows down till the Rio Ñorquin where it gets further diluted. Thanks to the input of hydrothermal waters, the pH and the temperature gradually vary downstream, from 70°C and pH 0.5 near the source, to 14°C and pH=7 when joining the Rio Ñorquin. These changes in physicochemical parameters make of Río Agrio a unique and extremely interesting natural environment for studying extremophilic microorganisms and their interactions with the habitat. For these and other reasons, Rio Agrio emerges as an environmental gradient model system in Latin America. Since 1997 our research group has studied the biodiversity and ecology of acidophilic microorganisms from this geothermal system and explored their biotechnological potential. Microbiological studies performed have focused on the characterisation and isolation of native acidophilic microorganisms by means of culture dependent and independent techniques setting the basis for deeper eco-evolutionary studies. The biodiversity detected at Río Agrio is higher than that of other acidic natural rivers or volcanic habitats analysed thus far, but lower than that presented on acidic mine drainage effluents making this system a highly tractable and appealing model system for community and population resolved studies. These pioneering molecular studies have paved the way for focused studies on the acidophilic prokaryotic communities composition and interactions, in the context of landscape ecology. As well, biological novelty uncovered in this unique environment is like to help improve or develop new biotechnological processes relevant for bioleaching and heavy metal bioremediation.

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Ecology and biochemistry of photoautotrophic microbial mat communities in a thermal gradient

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The microbial thermal mats represent ideal study models to understand the principles of microbial ecology and biogeochemistry. The effect of temperature in the microbial and viral communities of the photoautotrophic mats in Porcelana thermal system (North Patagonia, Chile) was investigated through integrative approaches: metagenomics, metatranscriptomics, proteomics and biogeochemistry. Its taxonomic composition showed dominance of bacteria (96%), with low abundance of Eukarya (0,5-5%) and Archaea (0,1-1%). In particular, phototrophic Cyanobacteria (*Mastigocladus* spp.) and Chloroflexi (*Cloroflexus* sp. and *Roseoflexus* sp.) dominate along the temperature gradient, with spatial replacement of these two primary producers, suggesting a niche differentiation by temperature. These groups co-occur at temperatures ~ 58°C, actively contributing to photosynthesis and CO_2. Three carbon fixation pathways are represented in the temperature gradient: Calvin-Benson (Cyanobacteria), bicycle-3-Hydroxypropionate (Chloroflexi) and Hydroxypropionate-hydroxybutyrate cycle (Archaea). Nitrogenase activity, $^{15}N_2$ cell uptake, and expression of the *nif*H gene, demonstrated that the incorporation of new nitrogen into Porcelana by N_2 fixation is due to cyanobacteria of the genus *Mastigocladus* (Order Stigonematales). Their contribution was approximately 3 g of N m^{-2} $year^{-1}$ and 27 g C m^{-2} $year^{-1}$, suggesting that these organisms completely cover the demands of these compounds in Porcelana. From this hot spring we isolated 82 strains that were morphologically and phylogenetical identified as thermal *Mastigocladus* spp. (99% identity in their 16S rRNA genes). These isolates form three different proteomic clusters unveiled by MALDI-TOF MS analysis, suggesting the presence of ecotypes along the thermal gradient. These thermophilic cyanobacteria could also be infected and then controlled by cyanophages of the order Caudovirales (representing up to 70% of total viral community). Full assembly of metagenomics reads led to the discovery of the first genome of a thermophilic Cyanopodovirus (TC-CHP58) associated with the thermophilic filamentous cyanobacteria *Mastigocladus* sp., with 25 to 48% of amino acid homology with other freshwater cyanopoviruses that also infect filamentous cyanobacteria. This work is a step forward to better understand adaptation and therefore evolution of thermophilic species of relevance for the biogeochemical dynamics in extreme systems.

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Microbial productivity in environmental gradients of Chilean aquatic systems

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Microbial communities in aquatic systems are crucial in the production and transfer of energy by intricate non-linear metabolic pathways through the food web. In this work, we assessed and discuss the biogeochemical contribution of natural microbial communities (as bulk primary and bacterial secondary production) from two isolated and extreme environmental zones from Chile, the Andean wetland Salar de Huasco (SH) and from the South Pacific Sub-tropical Gyre (SPG). A total of 11 stations for SH, and 10 stations (at two depths) for SPG were sampled along transects based on environmental gradients related to water origin and physicochemical changes. Collected water from each station was inoculated with $NaH^{14}CO_3$ and ^{3}H-leucine to obtain primary production (PP) and bacterial secondary production (BSP) estimates under natural solar radiation and *in situ* temperatures. Our results showed contrasting values in microbial production within the sampled stations. The highest contributions to daily PP and BSP were obtained in the most extreme environmental conditions (usually the most isolated and "stress exposed stations"). The patchy but high contribution of microbial bulk productivity to the global C cycle is novel for SH and the SPG. These remote Chilean ecosystems are hot spots not only for new extreme microbial biodiversity, but for higher organic matter production and remineralization (which are usually coupled) than the previously thought. Finally, priorities for future research are presented for open discussion from methodological constraints to environmental and economic implications.

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S08

Chasing RNA viruses: hosts, pathogenesis and spread

Chair: Dr. Nicole Tischler

New hantaviruses emerging in new hosts

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Hantaviruses (genus *Orthohantavirus*, family *Hantaviridae*), mainly considered as rodent-borne viruses, are important, globally emerging human pathogens causing hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome. Our view on hantavirus ecology and host range was fundamentally extended over the last decade; the dogmatic concept of hantaviruses as rodent-borne viruses changed after 2007 when more than 20 new hantaviruses were described in non-rodent hosts belonging to the mammalian superorder Laurasiatheria, including shrews and moles (order Eulipotyphla) and more recently also bats (order Chiroptera). After identification of Tanganya virus found in Therese's shrew (*Crocidura theresae*) trapped in Guinea, West Africa, our extensive molecular screening of shrews revealed the presence of several novel shrew-borne hantaviruses also in Central Europe. Newfound Seewis virus (associated with Eurasian common shrew, *Sorex araneus*) and Asikkala virus (associated with Eurasian pygmy shrew, *Sorex minutus*) showed strong geographic clustering as well as high genetic divergency. Moreover, opportunistic testing of a European moles (*Talpa europaea* revealed that at least two mole-borne hantaviruses, Nova virus and Bruges virus are present in Europe and associated with the same reservoir host. Because bats are recognized as one of the most considered important reservoir hosts for emerging human pathogens, we further extended our studies also to bats. Magboi virus was identified as the first bat-borne hantavirus in Slit-faced Bat (*Nycteris hispida*) from Sierra Leone, West Africa. Most recently, we found a novel hantavirus, provisionally named Makokou virus (MAKV), in Noacks Roundleaf Bat (*Hipposideros ruber*) in Gabon, Central Africa. Phylogenetic analyses including ancestral state reconstruction combined with phylogenetic fossil host hypotheses testing indicated the mammalian superorder Laurasiatheria (including shrews, moles, and bats) as potential hosts of ancestral hantaviruses.

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Pose novel rodent- and insectivore-borne hantaviruses a threat to human health?

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Very recently, novel hantaviruses were molecularly detected in rodents, insectivores (shrews and moles) and bats. Rodent-associated hantaviruses are known to cause hantavirus disease (hemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome) of different severity in humans. However, nothing was known whether hantaviruses associated with unconventional (non-rodent) reservoirs pose a threat for human health. In general, hantavirus infections in a human population can be diagnosed and typed by serological assays (ELISA, immunoblot, indirect immunofluorescence, focus reduction neutralization test) and molecular methods (nucleic acid amplification and sequencing) together with epizoonotical investigations. In my talk, I will give two examples how to assess the hazardousness of new viruses towards humans. We have isolated Sochi virus (belonging to the Dobrava-Belgrade virus species as a new genotype) from Black Sea field mouse, *Apodemus ponticus*, in South-East Europe. Clinical studies of a patient group with clearly demonstrated Sochi virus infection have shown that the virus causes moderate to severe hantavirus disease with a case fatality rate of about 15%. This qualifies Sochi virus as the most pathogenic (virulent) hantavirus in Europe (1). Moreover, by use of newly established serological assays, we show evidence for insectivore-borne hantavirus infections in humans from Côte d'Ivoire and Gabon (2). Acute and anamnestic human sera specifically reacted with antigens from Bowé virus, (hosted by Doucet's musk shrew, *Crocidura douceti*) or Uluguru virus (discovered in the Geata mouse shrew, *Myosorex geata*). Since exact genotyping of the viruses cannot be assured with the applied tests, one can conclude that the infections were caused by these viruses or by viruses serologically closely related to them.

(1) Kruger DH, Tkachenko EA, Morozov VG, et al, Life-Threatening Sochi Virus Infections, Russia.Emerg Infect Dis 2015; 21(12): 2204-8.
(2) Heinemann P, Tia M, Alabi A, et al, Human Infections by Non-Rodent-Associated Hantaviruses in Africa. J Infect Dis 2016; 214(10): 1507-11.

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Replication, assembly and spread of human metapneumovirus: Lessons learned from a recently discovered human pathogen

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Human metapneumovirus (HMPV) is a respiratory pathogen that causes significant upper and lower respiratory disease in all age groups worldwide. Currently, no effective antiviral therapy or vaccines are available against this pathogen. Considerable effort has been made to understand HMPV entry into cells, but the subsequent processes of

replication and assembly are less understood. After the surface viral glycoproteins mediate attachment and fusion with cellular membranes, the nucleocapsids are released into the cell cytoplasm to initiate transcription and replication of the viral RNA genome. In this work, we sought to answer basic questions about the specific sites and timing of replication, transcription, and viral assembly. Combining fluorescence *in situ* hybridization (FISH) with immunofluorescence and confocal 3D reconstructions, HMPV replication and transcription were imaged in human bronchial epithelial cells from very early times post-infection. Like other non-segmented negative-strand viruses, HMPV replication and transcription occurs in well-defined cytoplasmic inclusion bodies. These are predominantly located at the perinuclear area and are distinct from other cytoplasmic structures such as stress granules or P-bodies. Surprisingly, HMPV inclusion bodies are maintained at very low numbers independent of the MOI or the time post-infection. Disruption of actin polymerization by the use of cytochalasin D resulted in an increased number of small replicative sites and a decrease in transcription and replication of the viral RNA, as measured by RT-qPCR. We hypothesize that inclusion bodies result from the coalescence of multiple individual replicative sites. From the inclusion bodies, nucleocapsids are transported to intercellular extensions and viral filaments that bud from HMPV infected cells, a process dependent on actin, but not microtubule polymerization. Our data using high resolution microscopy and electron microscopy suggest that HMPV-induced intercellular extensions are a strategy used by the virus to disseminate directly from cell-to-cell. Furthermore, preliminary data using single cell fluorophore injection and live imaging suggest that HMPV components can be directly transmitted between the cytoplasm of an infected cell to a target cell. Our results highlight a pivotal role of the actin cytoskeleton in replication and assembly of HMPV, and also have important clinical implications such as the identification of new potential therapeutic targets.

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Hantavirus receptors expression in a lethal pulmonary syndrome animal model

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Hantaviruses are enveloped viruses which belong to the Bunyaviridae family. They are known to cause hemorrhagic fever with renal syndrome (HFRS) and the highly lethal hantavirus pulmonary syndrome (HPS) in humans. Both are related with an increased vascular permeability; yet several pathogenesis factors are still unknown. Actually, a differential receptor usage is under study. Receptors such as the □3 integrin subunit and the receptor for the globular head domain of complement C1q (gC1qR) have been described to be used by pathogenic hantaviruses in vitro. In this work, we analyze the expression of □3 integrin and gC1qR in an animal model for HPS. For this purpose, we performed immunohistochemical and qPCR studies of lungs and kidneys at 8 and 12 days post-infection (d.p.i.) with Andes hantavirus. In control and 8 d.p.i animals, both

receptors are localized in pulmonary veins while in 12 d.p.i. moribund animals the lungs exhibit strong features of edema accompanied by weaker receptors staining. Interestingly, the qPCR results show that the expression of □3 integrin and gC1qR is reduced in lungs of 8 d.p.i. animals, even though the histology of the tissue appears normal with no signs of disease. The decrease of receptors mRNA levels in the lungs at 8 d.p.i. seems to be highly specific since infected kidneys of the same animals show similar receptor mRNA levels compared to controls. Together, these results suggest that the reduced expression of □3 integrin and gc1qr receptors may be an important initial step involved in vascular permeability and subsequent pathogenesis.

FUNDING: CONICYT by grants FONDECYT 3150695, 1140050 and Basal PFB-16.

S09

Topics in virology: meeting the next generation of Chilean Virologists

Chairs: Dr. Marcelo López-Lasta and Dr. Rafael Medina

South America as corridor for the introduction of avian influenza virus into Antarctica

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We, and others, recently identified an H5N5 avian influenza virus (AIV) in chinstrap penguin in Antarctica containing North American and Eurasian lineage genes. It is unknown how this virus was introduced to Antarctica, and what are the natural reservoirs contributing to the movement of these IAVs. We have identified migratory species that are IAV positive mainly during their arrival to the Southern cone, such as: *Leucophaeus pipixcan*, *Rynchops niger*, *Haematopus palliatus*, *Calidris alba*, *Numenius phaeopus hudsonicus*, suggesting that IAV are imported yearly by migratory birds to South America. We have also determined a high level (51%) of seroprevalence to IAV in resident local wild ducks (Anas georgica) after the migratory season, suggesting the circulation of endemic strains in the region. Analyses of complete genome sequences obtained from three Leucophaeus pipixcan and one Leucophaeus modestus corresponding to the H13N2 subtype, showed that these gull viruses clustered closely with gull H13N2 AIVs from Chile and Peru from 2007-2010 and gull viruses from North America. We identified 3 internal segments (PB2, PA and NS) from the Antarctic H5N5 virus that grouped close to South American isolates. Of note is PA that grouped close to the PA from the gull viruses, suggesting a common ancestor and a plausible direct relation between South American and Antarctic viral genes. Our results provide important information of ecological niches contributing to the transmission and persistence of influenza viruses in the American and Antarctic continents.

Center For Research In Influenza Pathogenesis (CRIP) an NIAID-NIH Funded CEIRS Center HHSN272201400008C, and the grants Anillo de Investigación en Ciencia y Tecnología - PIA ACT1408 and FONDECYT Postdoctoral 3150564 from CONICYT Chile.

Clinical and molecular predictors of severity in Andes Hantavirus induced disease.

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Andes virus (ANDV) is the etiological agent of hantavirus cardiopulmonary syndrome in Chile. Individual host factors affecting ANDV infection outcome are poorly understood. Using a case-control genetic association analysis in 238 ANDV-infected patients we explored the link between single-nucleotide polymorphisms (SNP) in *il28B* am *tnf-a* genes and the clinical outcome of ANDV-induced disease. Analysis of IL28B SNPs rs12979860 and rs8099917 revealed a link between homozygosity of the minor alleles (TT and GG, respectively) and a mild disease progression, while heterozygosity or homozygosity for the major alleles (CT/CC and TG/TT, respectively) in both IL28B SNPs was associated with severe disease. No association with the clinical outcome of HCPS was observed for TNF-α SNP rs1800629 (TNF-308G>A). We also evaluated the profile of the pro-inflammatory cytokines IL-1β, IL-12p70, IL-21, TNF-α, IFN-γ, IL-10 and IL-6 in serum samples of ANDV-infected patients at the time of hospitalization. Significantly elevated levels of TNF-a, IFN-γ, IL-10, and IL-6 were detected in ANDV-infected individuals (p=<0.0001, 0.0036, <0.0001, <0.0001, respectively). Notably, IL-6 levels were significantly higher (40-fold) in the patients with severe symptoms compared to individuals with mild symptoms (p=<0.0001). We conclude that the serum level of IL-6 is a significant predictor of the severity of the clinical outcome of ANDV-induced disease. Therefore, we present compelling evidence suggesting that the knowledge of the host genomic background as well as cytokines expression profile at early stages of the disease allows predicting the possible outcome of an ANDV infection, offering the promise of personalized intervention.

Iniciativa Científica Milenio, Proyecto IMII **P09/016-F**; Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT) through Programa Investigación Asociativa (PIA) **ACT1408**.

Positive regulation of virus activated-innate immune signaling by the E3 ubiquitin ligase TRIM65

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Viral infection triggers a fast and effective cellular response mediated primarily by the production of INFβ that induce an anti-viral state through complex signal cascades. Therefore, the regulation of its induction and subsequent IFNβ signaling needs to be tightly controlled. There is growing evidence implicating the members of Tripartite-motif (TRIM) protein family of E3 ligases as critical players in this regulation. However, the exact role, mechanism of action, and the physiological relevance of their activity *in vivo* still remain poorly investigated. Previous work in our lab revealed that an unprecedented

large number of TRIMs play critical roles as enhancers in the regulation of innate immune signaling pathways. Our recent studies focused on TRIM65 have revealed that its overexpression strongly increased the 2CARD-RIG-I-dependent activation of the INFβ and ISRE promoters indicating that TRIM65 has a role in the interferon induction pathway. Consequently, IFNβ, ISG54, Mx1, IL-6 as well as OAS1 mRNA levels are decreased in TRIM65 knock down cells upon infection compared to infected/treated control cells. These data suggest a stimulatory role for TRIM65 in innate immune signaling. Besides, TRIM65 showed antiviral activity comparable to TRIM25 in a PR8-GLuc antiviral assay. Since the E3 ubiquitin ligase activity of many TRIMs has been linked to their antiviral functions, we have identified RIG-I, MDA5 and IRF3 as TRIM65 interacting factors and potential substrates. Our futures studies are focused on delineate the molecular mechanism by which TRIM65-mediated ubiquitin or ubiquitin-like modifications could regulate the response to viral infection. To study the role of TRIM65 in innate immune signaling we have used luciferase assays, Co-immunoprecipitations assays in 293T cells, transient knock down using siRNAs in 293T cells, Western blots, RT-qPCR, PR8-GLuc antiviral assays. A better understanding of positive regulatory networks of the IFN response will provide new knowledge that will help to design more effective therapeutics.

NIH/NIAID HHSN272201400008C

Clinical and immunological host factors associated to severe influenza A virus infection

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Influenza A virus infections cause seasonal epidemics and occasional pandemics, and remain a major cause of morbidity and mortality worldwide. Disease severity depends on virological and host factors, mainly due to extreme age, pregnancy, immunosuppression and numerous identified comorbidities; however, it is still unclear what specific molecular factors are at the basis of disease outcome in the general population. In the last 6 years, to address disease severity in the general population in Chile, we have established a clinical study to recruit influenza patients, to conduct a comprehensive analysis to begin understanding the host factors that affect clinical outcome. We analyzed clinical metadata from 128 severe and 94 non-severe patients, evaluated the status of the innate and adaptive immune responses by measuring pro- and anti-inflammatory cytokines during the acute phase of infection (days 1-4) using a Multiplex ELISA, and determined seroconvertion by hemagluttination inhibition assay at 21-28 days after infection. We also evaluated the immune genetic predisposition by evaluating single nucleotide polymorphisms (SNPs) of Interleukin 28B (*IL28B*; an

antiviral cytokine) and the Tumor Necrosis Factor-a (*TNF-a*; a pro-inflammatory cytokine) genes, which expression level depends on the presence of SNPs rs12979860 (CT/TT/CC) - rs8099917 (TG/GG/TT) and rs1800629 (GA/GG/AA), respectively. Our results to date show that risk factors like obesity (BMI>30) and chronic cardiovascular diseases (including arterial hypertension) are more prevalent in patients with severe infection. Also, we found a higher expression profile of the pro-inflammatory cytokines IL-6 and 8, during the acute phase of the infection, in severe compared to the non-severe patients; and a higher expression of the anti-inflammatory cytokine IL-10, associated with severe patients that required treatment at the intensive care unit to overcome the disease. On the other hand, this group also had a genetic predisposition to express higher amount of IL-28B. Meanwhile, no genetic predisposition for TNF-a nor seroconvertion rate was found to affect the clinical outcome to infection. These results expand our understanding of host factors contributing to the pathogenesis of influenza, which can have an impact on therapeutic and prophylactic interventions targeting those at higher risk severe disease.

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Role of the Andes Virus NSs protein in the modulation of host innate immunity.

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Andes virus (ANDV) is a rodent-borne hantavirus member of the *Bunyaviridae* family of viruses. ANDV is endemic in Argentina and Chile and is the major etiological agent of hantavirus cardiopulmonary syndrome (HCPS) in south America. ANDV features a tripartite genome consisting of three negative polarity single-stranded RNA segments designated large (L), medium (M) and small (S), packed into helical nucleocapsids. The L and M messenger RNAs (mRNAs) encode the RNA polymerase and a glycoprotein precursor that is co-translationally processed to yield two envelope glycoproteins (Gn and Gc), respectively. The SmRNA encodes the nucleocapsid (N) protein and the NSs protein from an overlapping (+1) open reading frame. At early stages of infection ANDV inhibits the cellular type I IFN response, through a yet not fully understood mechanism. In addition, the role of the ANDV-NSs protein remains unknown. Here, we will present data suggesting that the ANDV-NSs protein plays an important role in the inhibition of the type I IFN pathway.

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Isolation of the ribonucleoprotein complexes associated to the HIV-1 Rev protein during viral replication in T-cells

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Human immunodeficiency virus type-1 (HIV-1) is a member of the lentivirus genus of the Retroviridae family that infects and destroys CD4+ T lymphocytes and is the etiological agent of the Acquired Immunodeficiency Syndrome (AIDS). Once integrated into the genome of the infected cell, the host RNA polymerase II recognizes the viral promoter and drives the synthesis of one single messenger RNA molecule, the 9-kb genomic RNA (gRNA). Early during viral gene expression the gRNA undergoes alternative splicing generating a set of fully and partially spliced transcripts. Later on, the gRNA in its unspliced form is exported to the cytoplasm and used as an mRNA for the synthesis of the structural protein Gag and the viral enzymes. Gene expression from the gRNA is highly dependent on the viral protein Rev, which promotes nuclear export and translation of this viral transcript. Although the activity of Rev on nuclear export is achieved through interactions with the host karyopherin CRM1 and other associated factors including eIF5A or DDX1, the precise composition of the Rev-associated nuclear export mRNP has never been characterized during viral replication in T-cells. The function of Rev in translation is also expected to be exerted through the specific recruitment of host factors such as translation initiation factors yet the composition of this Rev-dependent translation initiation complex is completely unknown. This work represents the starting point towards the characterization of two specific ribonucleoprotein complexes associated to the HIV-1 Rev protein. For this, we first developed a Jurkat TRex cell line carrying the HIV-1 NL4.3-ΔRev provirus integrated in its genome. This T-cell line was then used to insert a tetracycline/doxycycline inducible Flag-Rev cassette. Our results show that both Flag-Rev and the Gag protein are synthesized only in the presence of tetracycline o dioxycycline. A kinetic study over 24 hours revealed that the peak of unspliced RNA versus Rev expression in the cytoplasm was reached at 8 hours post-induction. This system is currently being used to isolate and characterize the nuclear export and translation initiation ribonucleoprotein complexes associated to the HIV-1 Rev protein.

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S10

Antarctic microbial ecology

Chair: Dr. Beatriz Diez

Marine observatory of Chile Bay: a model study of coastal microbial ecology and biogeochemistry in Antarctica

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Chile Bay is located on Greenwich Island (South Shetland Islands) in the Western Antarctic Peninsula. This coastal marine system is a study model to better understand how climate change is affecting ecology and microbial biogeochemistry in this polar region. In this natural observatory we are studying changes in microbial and viral structure, composition and function in the austral summer since 2013. Some of these relevant functions that we are studying are, for example, the use of proteorhodopsin (PR) (proton pump promoted by light) or the assimilation and fixation of C and N by different metabolic pathways, strategies that have been little or never studied in Antarctic waters. On the other hand, the effect of both biotic and abiotic factors, and in particular those associated with deglaciation, on the taxonomy and function of microbial communities and their interactions, are the focus of our studies. We are using different "omics" methodologies (metagenomics, metatranscriptomics, metaviromics) for the characterization of these communities, accompanied by *in situ* experiments in the field that allow us to quantify the rates and microbial activities involved in the biogeochemical cycles. Our results reveal both taxonomic and functional differences depending on environmental factors such as salinity or climatic conditions such as wind, and that productivity in this marine system is resilient to the disturbance, suggesting that some members of the community are important indicators of deglaciation in these Antarctic coastal system. It was also revealed that in these coastal waters bacteria affiliated with alpha-, gamma-proteobacteria and bacteroidetes possess a very active PR gene throughout the day, being the first genetic evidence of PR-mediated photoheterotrophy in Antarctic waters. Additionally, we discovered new phages belonging to Caudovirales that infect bacteria, as well as viruses of both DNA and RNA that infect dominant components of phytoplankton (diatoms and haptophytes), from which their genomes were obtained. These viruses could play a relevant role in the decay of phytoplankton blooms, thus affecting biogeochemical cycles in the Southern Ocean. All these results represent a novel contribution, with a significant impact on the conceptual models of this polar marine region.

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Functional metagenomics of microbial communities associated to Antarctic sponges: diversity and functionality across the three domains of life in a poorly explored ecosystem.

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Marine sponges harbor a diverse, abundant and host-specific microbial community, composed of bacteria, archaea and eukaryotes. These communities perform fundamental processes related to biogeochemical cycling and nutritional coupling with the host. Despite the fundamental roles that sponge- associated microorganisms have shown, most studies about sponge holobionts, i.e. host–microbial assemblages, have focused on the bacterial component and come from temperate and tropical environments. This implies that general conclusions are far to be extrapolated as transversal to all sponge holobiont systems. In the Antarctic benthos, sponges can occupy up to 80% of the available substrate, thus playing a key role as ecosystem engineers in benthic communities structuring, however, the functional roles of sponge symbionts from Polar Regions remain largely unknown. Here, we use a combination of metagenomic and culture-based approaches, with the aim to explore the functional potential hidden in the vast and versatile microbial communities associated to Antarctic sponges. Our analyses indicate that there are different diversity and similarity patterns between bacterial/archaeal and eukaryote microbial symbionts. Antarctic sponge holobionts display a particular taxonomic signature, with a total absence of Cyanobacteria and Poribacteria, two commonly found phyla in temperate and tropical sponges. Functional potential analysis shows genes involved in the nitrogen, carbon, sulfur and phosphorus cycles. Protein-coding genes related to Vitamin synthesis and host-microbe interactions are enriched inside the analyzed sponges when compared to the planktonic communities. Genomic features of bacterial strains isolated from these Antarctic sponges were also addressed. Overall, Antarctic sponge microorganisms showed a metabolic potential related to nutrient cycling and display several signatures related to symbiotic lifestyle. To our best knowledge, this work is the first insight into the functional potential of sponge- associated microbial communities from the Antarctic environment and reveals that fundamental biogeochemical, defense and nutritional processes are transversal across sponge-holobionts. Further/upcomming? studies will allow us to provide specific functional elements that could distinguish Antarctic sponge microbiomes from those from elsewhere.

INACH RG_31-15 "DIVERSIDAD Y VARIABILIDAD INTERANUAL DE COMUNIDADES MICROBIANAS EUROCARIONTES EN AGUAS COSTERAS ANTÁRTICAS"

Anthropogenic pressure over the Antarctic microbial world: Stability of soil communities facing hydrocarbon pollution disturbance

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Abiotic limitations are prevalent in most Antarctic soils, with scarce or absent vegetation and a general lack of insects or herbivores. In this scenario, microorganisms are particularly relevant as they are the drivers of most ecological processes. In this simplified food web, nutrients cycling are dominated by the microbial world. Because of this relative simplicity, Antarctic soils are ecosystems especially vulnerable to disturbances. Human activities and settlements generate a major environmental risk due to pollution by waste management, liquid effluents, air pollutants and fuel leaks. Hydrocarbon pollution is an anthropogenic disturbance of major concern since abiotic limitations such as low temperatures and nutrient limitation slow down the natural microbial attenuation of HC. Environmental disturbances, either natural or anthropogenic, alter microbial communities (MC), affecting their stability and, eventually, their ecological functions. Therefore it is crucial to assess to what extent anthropogenic disturbances can impair Antarctic MC stability and ecological functions. Stability depends on the nature of the disturbance, the environmental physicochemical properties and the MC structure. Nonetheless, the actual impact of each factor is poorly understood and remains as a major challenge in microbial ecology. In the present research, hydrocarbon pollution (the disturbance model) over Antarctic soil MC (the system model) is being studied. The research is composed by two parts. The first involve the sampling of several soils from Antarctic Peninsula and South Shetland Islands, including polluted and non-polluted sites, in order to assess the environmental drivers of MC diversity/structure. The second is focused on the effect of the history of previous pollution events over the MC stability facing forthcoming oil spills. For this, controlled microcosm experiments emulating field conditions were mounted using soils with and without pollution history in order to study the resistance, resilience and hysteresis of MC. Community stability and dynamics are being studied mainly by 16S rRNA gene sequencing. Altogether, this research is expected to contribute with some clues to basic questions in microbial ecology. Patterns emerging from ecological parameters are expected to be useful as pollution/remediation indicators and, ideally, help to predict community response facing future oil spills threatening the Antarctic continent.

FONDECYT postdoctorado 3160424. FONDECYT regular 1150171. CONICYT DPI-20140044.

S11

Fungi in the food industry: risks and opportunities

Chair: Dr. Luis Castillo

Physiological and molecular study and control methods on the spoilage wine yeast *Brettanomyces bruxellensis*

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Brettanomyces bruxellensis has been described as the main yeast contaminant in wine, because it is able metabolized hydroxycinnamic acids, present in grape, to phenolic derivatives, with produced off flavour. These acids are a stress factor to the yeast. A comparative analysis of the transcriptome of two different strains was carried out, which has allowed us to propose a model of the response of *B. bruxellensis* on stress conditions. In presence of hydroxycinnamic acids, the yeas present a generalized stress in which the expression of proton and efflux of toxic compounds. This latter could be involved in the outflow of nitrogen sources such as amino acids or allantoin. In general, we observed a higher expression of oxidative stress genes. These genes have been validated in *Pichia pastoris* GS115, observed that the presence of a copy of the analyzed gene improves the growth rate compared to the untransformed strain grown in the same condition. On the other hand, in the elaboration of wines to avoid its growth, the must is commonly complemented with SO_2. However, the use of this compound has been questioned by the possible health problems that entail its consumption. In this sense, our laboratory has explored other alternatives as well as the use of natural antimicrobial: a) Strain *Candida intermedia* LAMAP1790 produces antimicrobial compounds and we evaluated their effect against *B. bruxellensis*. Using joint seed testing and viability after direct exposure to the *C. intermedia* sterile culture medium, the secreted compounds were found to have fungicidal activity on four *B. bruxellensis* strains. b) Extract of Chilean native plant (*Cestrum parqui*), it shows an antimicrobial effect on the growth of *B. bruxellensis* in white and red wine. In both studies of antimicrobial compounds, there was no effect on *Saccharomyces cerevisiae*. In addition, we have performed tests in relation to the effect of light on growth of *B. bruxellensis*, based in the observed in other filamentous fungi, to use as a control method. In this case, we observed that in general *B. bxuxellensis* respond to 2500 to 4000 lux, inhibiting their growth.

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Fungal LOV domains for optogenetic control of heterologous protein expression and flocculation in yeasts

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Optogenetic switches promote gene expression upon light stimulation. These synthetic switches have become in a powerful tool for gene expression, with potential uses in heterologous protein expression and phenotypes control, overcoming the obstacles of chemical inducers and replacing its uses by light. In this work, we implemented in yeast an optogenetic system based on the interaction of two blue-light photoreceptors from *Neurospora crassa*, WC-1 and VVD, which we called FUN-LOV system. The FUN-LOV showed over 1300-fold of induction for the luciferase reporter gene upon blue-light or white-light stimulation, and with a highly dynamic and temporal resolution. We used the FUN-LOV system for heterologous expression of the biotechnologically relevant enzyme, limonene synthase. Western blot analysis showed over a 100-fold of protein induction upon light activation, representing 2.5 times more expression than chemical inducer activation. Additionally, we used the FUN-LOV system to control the expression of genes related with yeast flocculation (*FLO1*, *FLO11* and *TUP1*). Depending of the target gene controlled by the FUN-LOV system, Flocculation in Light (FIL) or Flocculation in Darkness (FID) were achieved. The light controlled expression of *FLO1* showed FIL phenotype, whereas the light controlled expression of *TUP1* showed FID phenotype. Overall, the results confirmed the potential of this optogenetic tool to control two biotechnologically relevant phenotypes, heterologous protein expression and flocculation, which finally results in the development of new yeast strains with industrial applications

CONICYT/FONDECYT Nº 3150156, CONICYT/FONDECYT Nº 1171151 and MN-FISB NC120043

Quest of natural fungicides to control *B. cinerea* on sustanaible agriculture industry

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Botrytis cinerea, also known as "gray mold fungus", causes serious pre- and post-harvest diseases in at least 240 plant species, including agronomically important crops. This pathogen is necrotroph, i.e. induces host cell death resulting in serious damage to plants tissues culminating in rot of the plant or the harvested product. The broad host range of *B. cinerea* results in great economic losses not only during growth but also during storage and transport of the Chilean export products. Traditionally chemical control remains as the main way to reduce the incidence of gray mold. The use of botryticides is necessary to control the infection. However, in many countries the regulatory authorities have started to restrict the use of chemical pesticides due to environmental concerns, and generation of resistant isolates caused by indiscriminate use of these compounds. Consequently, development of new strategies to prevent infection by *B. cinerea* has become a matter of current interest. At the same time, the search for natural antifungal compounds with the ability to control *B. cinerea* is a challenge that has acquired great importance in the last years. Plants are an important source of secondary metabolites, standing out phenolic compounds which have shown activity against several fungi species. Recently, we have obtained various compounds from *Drymis winteri* Forst and *Eugenia caryoplyllata* that are able to affect the germination and hyphal growth in resistant Chilean isolates of *Botrytis cinerea*. In addition, the mechanism by which these compounds can control *Botrytis*, has been also elucidated. However, it is worth to mention that natural products are less persistent in the environment and have a lower "half-life" than synthetic fungicides. This is obviously a disadvantage in the application and development of natural products as an alternative in the treatment of *B. cinerea*, but its ecological impact is much lower. Because of this, the need arises to protect these compounds by encapsulating them within nanostructures that extend their useful life and at the same time increase their activity.

FONDECYT 11140194 and 1130742

NEW MEMBERS SESSION I

Chair: Dr. Renato Chávez

High throughput proteomic analysis of *At. ferrooxidans* biofilms on pyrite

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In acidic conditions, sulfide minerals, especially pyrite, generate toxic ROS, resulting in oxidative stress for acidophilic iron/sulfur oxidizing microorganisms. Reactive oxygen species (ROS) cause oxidative stress and growth inhibition by inactivation of essential enzymes, as well as DNA & lipids damage in microbial cells. Mineral grinding enhances their generation due to an increased surface area. Consequently, oxidative stress management is especially important in acidophilic leaching microorganisms. Several adaptive mechanisms used by acidophilic iron-oxidizing bacteria have been described, but the molecular repertoire of adaptive responses upon exposure to pyrite and the presence of ROS is not thoroughly understood. In this study we used shot-gun proteomics to elucidate the adaptive responses after biofilm formation by iron(II)-grown cells on pyrite grains of *Acidithiobacillus ferrooxidans* ATCC 23270^T. This was done by comparing proteomes from 5-day old biofilms against iron-grown cells, which were used as inoculum for biofilm formation experiments. In total, 1157 proteins were found in proteomes of iron(II)-grown cells and 5 day-old biofilm cells on pyrite. After statistical analysis for strong changes on abundance levels ($1 > \log2 > -1$, q-value ≤ 0.05), 420 proteins were selected. From this group, 213 and 207 proteins had increased levels in iron(II)-grown cells and in 5-day-old pyrite biofilm cells, respectively. Proteins involved in ROS degradation, redox balance, macromolecule repair mechanisms, metal and oxygen homeostasis had increased levels in biofilm cells. In addition a transition of cells adapting from oxidation of iron(II) ions as sole energy source to the oxidation of reduced inorganic sulfur compounds (RISC) as additional energy sources in pyrite cultures was observed in biofilm cells. This study provides new insights related to biofilm lifestyle and molecular responses against oxidative stress in *At. ferrooxidans*.

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The use of two culturing methods in parallel reveals a high prevalence and diversity of *Arcobacter* spp. in a wastewater treatment plant

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The genus *Arcobacter* belongs to the family *Campylobacteraceae* and includes species considered as emerging food and waterborne pathogens. The *Arcobacter* spp. are known to be present in water environments and have been linked to the presence of faecal pollution. However, only a few studies have investigated its prevalence in wastewater, in which the only isolated species were A*rcobacter butzleri* and *Arcobacter cryaerophilus*. This study aimed to establish the prevalence of *Arcobacter* at a WWTP using in parallel two culturing methods (direct plating and culturing after enrichment) and a direct detection by a m-PCR. In addition, the genetic diversity of the isolates was established using the ERIC-PCR genotyping method. *Arcobacter* spp. were found in 96.7% of the studied wastewater samples from which 651 isolates were recovered that belonged to 424 different ERIC genotypes or strains (65.1%). Only few strains (4/424) showed persistence in different sampling points of the WWTP or sampling dates. This could mean that the strains recovered in the present study were originated from many different sources or they present a high genetic variation. Despite the predominant species were *A. butzleri* (58.3%) and *A. cryaerophilus* (35.4%), six additional species were recovered *i.e*. *Arcobacter thereius* (2.1%), *Arcobacter defluvii* (1.9%), *Arcobacter skirrowii* (1.2%), *Arcobacter ellisii* (0.5%), *Arcobacter cloacae* (0.5%) and *Arcobacter nitrofigilis* (0.2%). The observed high prevalence and genetic diversity of *Arcobacter* spp. from wastewater confirms that this is an important reservoir for known and new species of this genus. On the other hand, the use of direct plating and culturing after enrichment in parallel allowed the recovery of more different species than in previous studies. Moreover, *A. cryaerophilus* was the predominant species by direct plating while after enrichment it was masked by *A. butzleri*. Therefore, the overall predominance of the later species could be considered as a bias associated with the use of the enrichment.

Pharmacological induction of heme oxygenase-1 impairs nuclear accumulation of Herpes Simplex Virus capsids upon infection

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Heme oxygenase-1 (HO-1) is an inducible enzyme that is expressed in response to physical and chemical stresses, such as ultraviolet radiation, hyperthermia, hypoxia, reactive oxygen species, as well as cytokines, among others. Its activity can be positively modulated by cobalt protoporphyrin (CoPP) and negatively by tin protoporphirin (SnPP). Once induced, HO-1 degrades iron-containing heme into ferrous iron (Fe^{2+}), carbon monoxide (CO) and biliverdin. Importantly, numerous products of HO-1 are cytoprotective with anti-apoptotic, anti-oxidant, anti-inflammatory and anti-cancer effects. The products of HO-1 also display antiviral properties against several viruses, such as the human immunodeficiency virus, influenza, hepatitis B, hepatitis C and Ebola virus. Here, we sought to assess the effect of modulating HO-1 activity over herpes simplex virus type 2 (HSV-2) infection in epithelial cells. There are no vaccines against HSV-2 and treatment options are scarce in the immunosuppressed, in which drug-resistant variants emerge. By using HSV strains that encode structural and non-structural forms of the green fluorescent protein (GFP), we found that pharmacological induction of HO-1 activity with CoPP significantly decreases virus plaque formation and the expression of virus-encoded genes in epithelial cells. Remarkably, we observed that once the virus entered target cells, accumulation of HSV capsids around the nucleus, which is necessary for viral genome delivery into this compartment was impaired in CoPP-treated cells, as compared to untreated cells. Furthermore, we observed that treating cells with a CO-releasing molecule (CORM-2) recapitulated some of the anti-HSV effects elicited by CoPP. Taken together, these findings indicate that HO-1 activity blocks the replication cycle of HSV in epithelial cells by impairing capsid accumulation at the nuclear periphery and that many of the antiviral effects can be recapitulated by CO.

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What does *Oenococcus oeni* eat when is under ethanol stress conditions?

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Wine quality is determined by its aroma and flavor profile, which can be improved to includes malolactic fermentation (MLF) in winemaking. MLF allows wine deacidification and increments flavor complexity and microbiological stability. This process consists on the decarboxylation of L-malic acid into L-lactic acid, conversion that decreases wine acidity. *Oenococcus oeni* is the main bacterial species that carries out the MLF, due to its ability to grow under the harsh conditions present in wine. However, *O. oeni* is not always able to achieve this task under these hostile conditions, often generating sluggish or stuck malolactic fermentations. For this reason, this process is considered one of the most difficult to manage during winemaking. Several studies have been carried out with the aim of understanding the metabolism of *O. oeni* under winemaking conditions; however, MLF still remains mostly unpredictable. In wine, ethanol is considered the main stressor, because it can injure cell membrane integrity and impact cell viability. Most studies on *O. oeni* survival in wine have focused on the deleterious effect of ethanol on the integrity of cell membrane and changes in the cell wall composition. Nevertheless, the central metabolism of *O. oeni* under winemaking conditions is still scarcely understood. In this work, we took advantage of the recently constructed and validated genome-scale metabolic model (GSM) of *O. oeni* PSU-1 strain (named iSM454) to predict the metabolic behavior and the nutritional requirements of *O. oeni* at different phases of its growth. The results clearly indicate that different nutritional requirements of the PSU-1 strain are necessary when ethanol concentration increases.This work contributes to a better understanding of *O. oeni* metabolism under oenological conditions, as well as to identify essential nutrients required under stress conditions, such as high ethanol content.

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NEW MEMBERS SESSION II

Chair: Dr. Renato Chávez

Comparison of *Piscirickettsia salmonis* infection in salmon-derived cell line cultures and zebrafish primary cell cultures

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Piscirickettsia salmonis is a bacterial fish pathogen known to cause major salmonid mortality in Chile. This Gram-negative gamma-Proteobacteria is a facultative intracellular pathogen and the etiological agent of piscirickettsiosis, a systemic disease that is characterized by acute anemia, liver and spleen nodules, septicemia and death. The bacterial virulence mechanisms and host-pathogen interactions during infection are yet to be elucidated. In this work, we proposed the use of zebrafish as a model to evaluate the fish innate immune response towards *P. salmonis*, compared with the response in salmon derived cell lines, the common model used to study this bacterium. First, zebrafish (*Danio rerio*) larvae were used to establish *P. salmonis* infection via circulatory injection. Zebrafish survival and neutrophil recruitment were altered in the presence of the bacteria. Afterwards, primary cell cultures derived from zebrafish kidney were exposed to the bacteria and compared to infections in two salmon cell lines (SHK-1 and ASK). *P. salmonis* exposure caused decreased cell viability in the tested cell cultures, but with different magnitude. The bacteria were observed by immunofluorescence staining for at least 5 days in the infected primary cell cultures, and 12 days in the cell lines. A differential immune cellular response after bacterial exposure was observed in both cell cultures. The salmon-derived cells showed a marked overexpression of IL-8, IL-10 and IL-12, and a decreased expression of IFN-γ, and the opposite was observed in the zebrafish-derived cells, as revealed by RT-qPCR. On the other hand, bacterial expression of genes related to Dot/Icm secretion system and toxins were increased during infection in both cell cultures. In summary, *P. salmonis* can infect primary cell cultures derived from zebrafish altering its survival and immune response. Cytokine expression were different in primary cell cultures compared to the salmon cell lines, which correlates with the presence of the bacteria in the cultures, and the expression of bacterial virulence factors in both types of cultures. This suggests that the repertoire of virulence factors used by *P. salmonis* to infect different fish cells is similar, but the cell types respond differently towards this pathogen.

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Effect of pathogenicity island excision in the colonization ability of *Salmonella enterica* serovar Enteritidis in mice

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Pathogenicity island excision is a phenomenon that has been described in several serovars of *Salmonella enterica* subspecie enterica. ROD21 is an excisable Pathogenicity Island found in the chromosome of *S.* Enteriditis that harbors several genes encoding virulence associated proteins. Our previous studies have shown that ROD21 excision may play a role in the ability of *S.* Enteritidis to cause systemic infection in the mouse, because strains unable to excise ROD21 show reduced ability to colonize liver and spleen. In this work, we determined the dynamic of ROD21 excision in vivo across the stages of the infective cycle performed by *S*. Enteritidis in mice. After different times post infection, bacterial load and excision frequency was determined in the gut and in internal organs. We observed that *S*. Enteritidis colonize the gut during the first 24h, could be detected at 6h on mesenteric lymph nodes (mLN) and appear on internal organs, such as spleen and liver, after 48h. We observed that the excision of ROD21 is a dynamic process across the infective cycle in the evaluated organs. Interestingly, on mLN, the bacterial population had the high rates of ROD21 excision of all evaluated organs, during the first 96 h post-infection but contrary to that, excision remains lower or undetectable on liver and spleen at this time, increasing drastically after 14 h post infection. In contrast a mutant strain, unable to excise ROD21, has a severe defect to colonize deeps organs after 96 hours postinfection.

CONICYT Beca Doctorado Nacional 21140116-FONDECYT Regular: 1170964-Millennium Institute on Immunology and Immunotherapy

IL-10 production during *Klebsiella pneumoniae* ST258 infection induce an anti-inflammatory environment required for host survival.

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Carbapenem-resistant *K. pneumoniae* sequence type 258 (CRKPN-ST258) are a major concern of public health worldwide. Together with antimicrobial resistance, these pathogens have also acquired strategies to evade neutrophil-mediated clearance. It has been described that during CRKPN-ST258 there is a fast recruitment of monocytic myeloid derived suppressor cells (M-MDSCs) to the airways, establishing an anti-inflammatory environment that delay bacterial clearance. Interleukin-10 (IL-10) production is one of the main suppressive mechanisms exploited by these cells. To evaluate whether IL-10 production by M-MDSCs is a main suppressive mechanism of the immune response during CRKPN-ST258 infection, IL-$10^{-/-}$ and C57BL6 WT mice were infected with 1 x 10^8 of a highly prevalent strain of CRKPN-ST258 strain (KP35), isolated from New York City. IL-$10^{-/-}$ mice presented an elevated mortality rate at day 7 as compared with WT mice, higher bacterial burden in the airways and higher lung damage, suggesting that the maintaining of an anti-inflammatory environment by IL-10 is a key suppressive mechanisms during CRKPN-ST258 infections. IL-10 can be produced by different immune cells, to evaluate whether IL-10 it has been produced by M-MDSCs, IL-10/GFP transgenic mice were infected intranasally with KP35 and the production of IL-10 was evaluated by flow cytometry at several time points post infection. Bacterial burden in lungs was evaluated to correlate the cellular infiltrate with the presence of bacteria. Neutrophils were the most important source of IL-10 at 24 and 48 hpi; however after 48 hpi, M-MDSCs replaced neutrophils as the most important IL-10 source. Therefore, our data demonstrate that in vivo, M-MDSCs are active IL-10 producers during CRKPN-ST258 infections. Future research is needed to determine whether early IL-10 producing neutrophils exert an effect on the development of the immune response against CRKPN-ST258 infections.

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Characterization of the ICE*Afe*1 transferosome present in the extreme acidophile *Acidithiobacillus ferrooxidans* ATCC 23270

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Dispersal of some mobile genetic elements depends on Type IV Secretion System (T4SS). In this work, we characterized the *tra*-type T4SS nanomachines present in the publicly available genome *Acidithiobacillus ferrooxidans* ATCC 23270, analyzing their organization, conservation, expression and the formation of the conjugative bridge. The system is composed of 5 genetic clusters encoding all required functions for the formation and stabilization of the pili, and for DNA processing. Organization of the clusters is well conserved with respect to other described mobile genetic element (plasmids and integrative and conjugative elements, ICEs). Conservation, organization and distribution of *tra* system present on the ICE*Afe*1 suggests it could have a role in the horizontal gene transfer of the element between related bacteria. Analysis of the occurrence of T4SS in other Acidithiobacilli showed that the *tra*-type is less frequent than *trb*-type in this taxon. The T4SS genes encoded on ICE*Afe*1 are transcriptionally active and their mRNA levels increase in response to mytomycin C treatment, which has shown a stimulatory effect on the excision and transfer of others ICEs. Also, using epifluorescence microscopy and pilin specific antisera we were able to detect the formation of conjugative "pili" at the cell surface of *At. ferrooxidans* ATCC 23270. The findings reported here constitute a contribution to knowledge of mobile genetic elements of environmental acidophilic bacteria, and pave the way for further studies on horizontal gene transfer of this group of bacteria.

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Put your models to work: two tales of the use of modeling for insights into microbial physiology and ecology in anaerobic environments

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Mathematical modeling is increasingly becoming a widespread tool for microbiology and biotechnology. In the past, this tool integrated biochemical and physiological data towards to enhance the understanding of microbial cell behavior under controlled environmental conditions in a defined media. Afterwards, the "genome sequencing boom" has provided the possibility to reconstruct cell-metabolic networks allowing to integrate genomic, transcriptomic, proteomic, and metabolomic information into predictive models that efficiently resemble the individual cell metabolism as a response of the dynamically changing environmental conditions. Furthermore, with the advent of the metagenomic shotgun sequencing combined with computational analysis, research on microbial and viral communities from a wide range of ecosystems provides an invaluable tool for ecological research.

In this work, we explore the results obtained from two examples. The first case study, based on the genomic information of pure culture coupled to experimental data, were incorporated into a genome-scale metabolic model designed to quantitatively explore energy conservation from acetate oxidation coupled to sulfate reduction in the sulfate-reducing bacteria, *Desulfobacter postgatei*. The second case study, based on a metagenomic information, aimed to investigate the relative abundance and prevalence of viruses in a wide range of aquatic biomes and further hypothesized their virus–host relationship.

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ORAL SESSION I

Chairs: Dr. Claudia Saavedra and Dr. Francisco Remonsellez

Transcriptional and post-transcriptional regulation of *ompX* from *Salmonella enterica* serovar Typhimurium under H_2O_2 stress

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During its infective cycle, *Salmonella* is phagocytized by immune cells, where it is exposed to adverse conditions, being oxidative stress one of the most important. In this oxidative microenvironment, *S.* Typhimurium survives by modulating gene expression through the action of transcription factors such as ArcA and MarA. Also, it knows another type of genetic regulation through small RNA molecules (sRNA) where regulates the expression of a gene. Studies in our laboratory through microarray, have been identifying a genetic regulation on various outer membrane proteins (Omp) where the *ompX* is one of them. In *E. coli*, the *ompX* gene is regulated by MarA transcriptional factor and in our laboratory, we have identified that ArcA also participates in this regulation; in other studies, have identified two sRNAs *micA* and *cyaR* over the *ompX* transcript in *E. coli*. We hypothesized that the *ompX* gene is regulated at the transcriptional level by ArcA and MarA and at the post-transcriptional level by sRNAs under H_2O_2 stress in *Salmonella*. By qRT-PCR and EMSA analysis, we show that *ompX* is downregulated by ArcA and MarA in response to H_2O_2 through a direct interaction with the promoter region, and also that ArcA upregulates the expression of *marA*. Increased promoter activity and *ompX* transcript levels did not correlate with increased protein levels under oxidative stress suggesting a post-transcriptional regulation. To elucidate this regulation of *ompX* mRNA in response to oxidative stress, we performed a bioinformatics analysis and we noticed a putative binding site of the sRNA *oxyS* in the *ompX* mRNA. To evaluate the post-transcriptional regulation, we used transcriptional and translational fusions that included the 5'-UTR regions of *ompX* mRNA, under the control of the pBAD promoter. The results showed that the sRNA *cyaR* regulates the stability of the *ompX* mRNA and that *micA* and *oxyS* participate in maintaining OmpX translation under oxidative stress. In accordance with our results, we show that in *S.* Typhimurium exposed to H_2O_2, *ompX* is regulated at the transcriptional level by ArcA and MarA, and at the post-transcriptional level by *cyaR, micA*, and *oxyS* sRNA.

FONDECYT 1160315

Comparative genomics analysis reveals the diversity of toxin-antitoxin systems in *E. coli* isolated from Crohn's disease patients. New insight associated to this emergent *E. coli* patotype

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Crohn's disease (CD) is a chronic inflammation of the gastrointestinal tract, thought to result from interactions between resident microbes and the host innate immune system, in genetically susceptible individuals. Changes in mucosa-associated microbiota have been observed in CD patients showing an increase in the frequency of adherent-invasive *Escherichia coli* (AIEC), as compared to healthy individuals. In consequence, AIEC has been proposed as a possible agent trigger for CD. AIEC are defined by their abilities to adhere to and to invade epithelial cells, and to survive and replicate within macrophages. Mechanisms that allow intestinal epithelium colonization, and host factors that favor AIEC persistence have been partly elucidated. However, bacterial factors involved in AIEC persistence are currently unknown. Toxin-antitoxin systems (TA) are recognized factors involved in bacterial persistence. They are small genetic elements, diverse in sequence, antitoxin nature and mechanistic, distributed in plasmids and chromosomes from bacteria and archaea. In addition to persistence, stabilization of mobile genetic elements is one of their remarkable roles. The aim of this study was to compare AIEC and non-AIEC strains isolated from CD mucosal biopsies using comparative genomics, to define their TA repertoire along with other genetic markers. Whole-genome sequences of 43 *E. coli* strains were analysed *in silico* for bacterial typing and to explore the virulence factors, antibiotic resistance genes, and plasmid content. The TA repertoire was explored using an *in house* TA database, and a comparison of their genetic contexts was done. The strains were phylogenetically heterogeneous with B2 being the main phylogroup. Plasmid replicons were identified in almost all strains, and bla_{TEM}, *strA-B*, *sul1-2* were the predominant antibiotic resistance genes. The TA repertoire was diverse, with 26 TA identified in the reference strain AIEC LF82, that are absent or located in different genomic contexts in certain isolates. Some TA are harbored by likely mobile genetic elements encoding virulence factors. We hypothesize that those TA could contribute to AIEC pathogenicity through the maintenance of these elements. The HipAB system, close to a fimbrial operon, and the MazEF/YoeB-YefM systems, close to the *pdu* operon, are examples that will be discussed in this work.

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Heterologous expression of the genome of hypovirus CHV1-EP713 in *Botrytis cinerea*: transformation with *Agrobacterium tumefaciens* and evaluation of changes in the fungal phenotype

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Botrytis cinerea is a phytopathogenic fungus that causes the gray mold disease in a wide host range, including flowers, vegetables and fruits. On the other hand, *Cryphonectria parasitica* also is a phytopathogenic fungus that infects the american chestnut, producing a mortal disease known as chestnut blight. Previously the CHV1-EP713 virus was isolated from *C. parasitica* and classified as hypovirus, given its ability to decrease the fungal virulence. Our aim was to express the CHV1-EP713 cDNA in *B. cinerea* and to determine if the production of mycoviral genomes causes alterations in the fungal phenotype. We used the pXH9 vector, containing the cDNA corresponding to the CHV1-EP713 hypovirus genome, and the p18 plasmid, containing the *vir* genes from the *Agrobacterium tumefaciens* Ti plasmid, for the pXH9-18 vector construction. Then we transformed *A. tumefaciens* cells and the recombinant clones were used to transform the fungal virulent strain, *B. cinerea* CCg55L. The nucleic acids of *B. cinerea* clones that presented resistance to the Hygromycin B, were analyzed by CF11-cellulose chromatography to determine the presence of dsRNA. We found a dsRNA molecule with an approximate size of 13 kilobase pairs (kbp), which corresponds to the size of CHV1-EP713 genome, suggesting that the CHV1-EP713 virus was detected. Our results revealed that *B. cinerea* CCg55L was transformed and the viral cDNA was expressed. The generation of replicative intermediaries of mycoviral genome strongly suggests that infection was established. Additionally, the phenotypic analysis showed that the transformed fungus present lower rates of radial growth and sporulation, in comparison with the virulent original strain of *B. cinerea*. Furthermore, *in vitro* assays performed in grapevine leaves, showed a decrease in the damage on the plant tissue inoculated with the fungal clones, in comparison with the original strain, suggesting a lesser virulence of these transformants. Finally, we observed differences in the oxidative response of the plant tissue when spores were used to infect grapevine leaves. In conclusion, we transformed a virulent strain of *B. cinerea* with the cDNA of the hypovirus CHV1-EP713, and its expression and/or mycovirus genome replication could be related with the changes observed, suggesting an hypovirulence phenotype.

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Polypyrimidine tract-binding protein (PTB) isoforms modulate Dengue virus mRNA translation

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Denguevirus (DENV), member of *Flavivirus* genus of the *Flaviviridae*, is a simple-strand positive RNA virus and etiological agent of Dengue, an emerging arthropod-borne infectious disease. Translation of the DENV mRNA occurs mainly in a cap-dependent and poly (A) independent manner. Polypyrimidine tract-binding protein (PTB), an RNA-binding protein with four RNA recognition motifs (RRMs), stimulates DENV replication. In this study we sought to determine if the influence of PTB on DENV replication was exerted at the level of mRNA translation. For this we evaluated the impact of PTB isoforms, PTB1, PTB2 and PTB4 on translation of a virus-like mRNA. Results show that PTB1 promotes protein synthesis from the virus like mRNA. In contrast PTB4 reduces translational of the virus like mRNA, while PTB2 overexpression had no effect on virus protein synthesis. Mutational analysis revealed that RRM1/RRM2 or RRM3/RRM4 mutations influence translation of the virus-like mRNA differently when evaluated in the context of PTB1 or PTB4. Therefore, we conclude that PTB isoforms differentially modulate DENV mRNA translation.

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Allelic variants of the GTR1 gene affect nitrogen transporters gene expression and nitrogen consumption in *Saccharomyces cerevisiae* during alcoholic fermentation

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Nitrogen is the second nutrient assimilated by yeast during alcoholic fermentation, its consumption is key for *Saccharomyces cerevisiae* to carry out this process. Deficiencies in nitrogen sources are one of the main problems in the wine industry. Studies in this area have shown that different strains of *S. cerevisiae* present different levels of ammonium and amino acid consumption during fermentation. The TORC1 signaling pathway is responsible for detecting nutritional signals and to coordinate cell growth. Among its targets are genes related to nitrogen transport and metabolism, however, it is unknown how TORC1 can impact on the different strains generating differences in nitrogen consumption. Previous studies have highlighted the *GTR1* gene as a candidate to explain differences in nitrogen consumption. The *GTR1* gene encodes a GTPase participating in the EGO complex responsible for stimulating TORC1 in response to the absence of amino acids. Therefore, different alleles of this gene could differentially affect the expression of target genes of TORC1 and consequently, impact on the nitrogen consumption. The objective of this work was evaluating the effect of allelic variants of *GTR1* on the differential expression of nitrogen transporters and the nitrogen consumption in *S. cerevisiae* strains during alcoholic fermentation. The *GTR1* gene was selected from QTL mapping performed to a tetraparental population whose parents belong to four distinct clusters: Wine/European, West African, North America and Sake. These allelic variants were validated by reciprocal hemizygosity assay, which were fermented in synthetic must and determined the nitrogen content by HPLC. Expression of *MEP1, MEP2, MEP3, GAP1, AGP1, DIP5, GNP1* and *TAT2* by qPCR at three points of the alcoholic fermentation was evaluated. Differences were observed in the consumption of ammonium, arginine, glutamic, serine, threonine, alanine and glutamine when comparing the Wine and West African alleles. In general, the strain with West African allele showed lower expression of the genes evaluate during the first hours of the fermentation process, which could explain the differences in the consumption of the nitrogen sources. In conclusion, the West African allele of *GTR1* affects the expression of the nitrogen transporters, possibly by a constant activation of TORC1.

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Contribution of Natural Killer T cells to the pathology caused by the Respiratory Syncytial Virus and the Metapneumovirus

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Background. Natural killer T (NKT) cells are unconventional T cell lymphocytes. The NKT cell receptors recognize glycolipids bound to the MHC-I-like CD1d molecule. The synthetic α- Galactoceramides (α-Galcers) loaded onto CD1d has been shown to be a potent NKT cell activator. Here, we evaluate the susceptibility to both hRSV and hMPV infections in $CD1d^{-/-}$ mice. **Methods.** BALB/cJ $CD1d^{+/+}$ mice and BALB/cJ $CD1d^{-/-}$ mice were challenged either with hRSV or hMPV. Three days post-infection, mice were euthanized and their lungs and the bronchoalveolar lavage fluid were recovered for cytometry and viral load and serum for detection of cytokines. **Results**. A significant decreased of neutrophils and eosinophils, reduced viral load, higher levels of IFN-γ and reduced DCs 103+ were found in hRSV- but not in hMPV-infected $CD1d^{-/-}$ mice as compared to hRSV-infected $CD1d^{+/+}$ mice, being similar to the mock-treated mice. To better understand as to how NKT cells modulate T cell immunity during these infections, DCs were infected with hRSV or hMPV, pulsed with different concentrations of α-Galcer and co-cultured with NKT cells. While stimulation by hRSV-infected DCs led to reduced levels of IL-2 secretion by NKT cells, hMPV-infected DCs promoted a significant IL-2 secretion by NKT cells as compared to mock-treated DCs. Further, the observed increase of IL-2 by NKT cells stimulated with hMPV-infected DCs was α-Galcer dose dependent. **Conclusions**. Our data suggest that $CD1d^{-/-}$ mice are more resistant to hRSV- but not hMPV- infection, thereby NKT induced by hRSV may be detrimental for the outcome of the infection. Thus, hRSV may be using NKT cells as a mechanism of evasion of host immune system.

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ORAL SESSION II

Chairs: Dr. Cecilia Toro and Dr. Mario Tello

Identification of genes determining survival within macrophages and virulence of adherent invasive *Escherichia coli* strains

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Adherent Invasive *Escherichia coli* (AIEC) are defined by the *in vitro* capacity to adhere and to invade intestinal epithelial cells and also to survive and replicate within macrophages. AIEC have been frequently isolated from patients with Crohn´s disease, a chronic inflammatory bowel disease. Although some adhesins/invasins have been found in some AIEC strains, virulence factors determining their survival into macrophages have not yet been identified. A bioinformatic comparative analysis allowed us to identify genes present in AIEC strains but absent in non-pathogenic strains of *E. coli*. These genes include loci encoding systems for biosynthesis of capsule (*kps*) and the siderophore yersiniabactin (*ybt*). In the other hand, we have used *Galleria mellonella* larvae as a model for *in vivo* AIEC infection*. G. mellonella* (wax moth) has been recently used as a model for the study of other human pathogens and the results suggest a correlation with the pathogenicity displayed in mice. In addition, wax moths can be maintained at 37°C, temperature at which most bacterial pathogens express their virulence factors. **Objectives.** To determine if the *kps* and *ybt* loci are expressed by AIEC inside macrophages, if they are required for survival/replication within them and if they influence the AIEC virulence *in vivo.* **Methods.** The expression of AIEC genes within macrophages was analyzed at different infection times by quantitative PCR. Subsequently, the genes were deleted in a clinical isolate and bacterial survival/replication were measured by amikacin protection assays. *G. mellonella* larvae were evaluated as a model for infection of AIEC, the number of dead larvae and LD_{50}s were calculated. **Results.** The mutations introduced at the *kps* and *ybt* loci caused a significant decrease in the AIEC capacity to survive and/or replicate within murine macrophages after 24h of infection. *G. mellonella* larvae are susceptible to AIEC infection. The larvae mortality is dose dependent, and the AIEC LD_{50} at 24h is significantly lower than the LD_{50} of a non-pathogenic *E. coli* strain. **Conclusions.** AIEC loci *kps* and *ybt* are required for survival/replication within murine macrophages. This is the first report in which *G. mellonella* is proposed as an *in vivo* model for AIEC infection studies.

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Infectious pancreatic necrosis virus (IPNV) enters CHSE-214 cells via macropinocytosis

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Infectious pancreatic necrosis virus (IPNV) is the etiologic agent of infectious pancreatic necrosis, which affects several salmonid species. IPN causes high mortality and produces great economic impact in salmon-farming countries. IPNV is a small, icosahedral non-enveloped particle that contains bipartite double-stranded RNA (dsRNA) genome and belongs to the *Birnaviridae* family. To date, little is known about the early events of IPNV replication such as the nature of the IPNV receptor or the pathways used for cell entry. The objective of this study was to determine the internalization mechanism used by IPNV to enter salmon cells. We blocked the molecular components associated to different endocytic pathways using specific inhibitors. We evaluated the effect of each treatment on virus infection in CHSE-214 and SHK-1 salmon cells. The IPNV infection was monitored by immunofluorescence assay using anti VP2/VP3 IPNV antibody and epifluorescence microscopy. The functionality of each endocytic pathway was evaluated using specific fluorescent tracers and visualized using laser scanning confocal microscopy. It was observed that IPNV stimulated the fluid phase uptake and virus particles co-localized with the soluble dextran-Texas red tracer (Dx-TR) after internalization. In addition, changes in the distribution of F actin were observed after inoculation of the virus and the infection was blocked with the disruption of actin dynamics with cytochalasin D. Notably, viral internalization was significantly reduced when the Na+/H+ anti-porter bomb NHE-1 was inhibited with 5- (N-ethyl-N-isopropyl) amyloride (EIPA). Taken together, these data indicate that IPNV enters the cells by macropinocytosis. To examine the role of macropinocytosis regulators, additional inhibitors were tested. The results suggest that IPN uses different cellular factors for its entry into the two cells lines used. Thus, in CHSE-214 cells, the requirement for Rho Rac1 GTPase, PAK-1 kinases, PKC and motor protein myosin II was observed. On the other hand, infection in SHK-1 cells showed dependence on GTPase Ras, GTPAsa Rho Cdc42, PKC and myosin II. Notably, the entry of IPNV in both cells types was resistant to wortmannin, suggesting the independence of PI(3)kinase.

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The transcription factor ArcA modulates in part the ability of *Salmonella* Typhimurium to survive inside neutrophils

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Neutrophils are included in the host´s initial response to pathogens, several microorganisms have evolved mechanisms to evade the microbicide action to survive and ultimately achieve systemic infection. In this context *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is a Gram-negative and intracellular pathogen that has several specialized virulence trades that enables it to thrive inside the phagolysosome and even replicate inside these compartments. Inside these phagocytic cells, *Salmonella* faces different kinds of stresses; among the most relevant we find the highly toxic Reactive Oxygen Species (ROS), which inside neutrophils is mainly hypochlorous acid due to the myeloperoxidase action. We have found that the ArcAB two-component system is involved in ROS resistance to both hydrogen peroxide and hypochlorous acid through transcriptional regulation, and aimed to investigate the extent of the role that ArcA has over the ability to survive the oxidative burst inside the *Salmonella* containing vacuole. In this context we compared the conditions from the highly described macrophages with our model inside neutrophils and found no significant difference in total ROS, however there is, as expected, different concentrations of hydrogen peroxide, additionally to further evaluate this phenomenon we measured the expression of several genes with functions related to detoxification, metabolism and virulence and found that ArcA has a role in regulating several genes in the infection context.

FONDECYT 1160315

The presence of m^{6}A within the 5´-UTR of the HIV-1 genomic RNA defines its use as mRNA or as the packaged genome

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The human immunodeficiency virus type-1 (HIV-1) genomic RNA (gRNA) is used as a messenger RNA for the synthesis of the structural protein Gag and as the viral genome packaged into newly synthesized particles. Previous *in vitro* analyses aimed to understand how these two mutually exclusive functions of the gRNA are regulated showed that the 5´-untranslated region (5´-UTR) acts as a riboswitch adopting two different structures that favor translation or packaging. However, studies using gRNA obtained from cells and viral particles showed no differences in the structures of the 5´-UTR suggesting that structural changes do not explain how the transition from translation to packaging occurs and thus, the regulation of this crucial step for the assembly of infectious particles is still uncertain. Interestingly, it was recently reported that HIV-1 contains the post-transcriptional modification N6-methyladenosine (m^{6}A) along its gRNA. m^{6}A was shown to induce degradation of the incoming gRNA (when acting as a genome) but to promote Gag synthesis (when acting as mRNA). These data prompted us to analyze the impact of m^{6}A in the transition from translation to packaging of the HIV-1 gRNA. We observed that overexpression of the m^{6}A methyltransferase complex resulted in increased Gag levels in a manner dependent on the catalytic activity of METTL3, suggesting that the synthesis of the viral protein is positively regulated by m^{6}A. Interestingly, we observed a decrease in the gRNA and Gag present in purified viral particles indicating a negative effect of m^{6}A in viral particle production. Strikingly, mapping the m^{6}A residues revealed the presence of methylations within the 5-UTR of the cytoplasmic gRNA but not in the virus-associated gRNA. Bioinformatic predictions suggest A198 and A242 as the methylated residues. Both adenosines are highly conserved amongst viral isolates and A242 was shown as a binding site for the NC domain of Gag, a key interaction required for the packaging of the gRNA. Taken together, our data suggest that the presence of m^{6}A in the 5´-UTR of the gRNA favors Gag synthesis in the cell but inhibits the production of viral particles probably by interfering with the binding of Gag.

FONDECYT 1160176

Genetic deletion of glycoprotein D in Herpes simplex virus type 2 enables *in vitro* and *in vivo* T cell activation by dendritic cells

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Herpes simplex virus type 2 (HSV-2) is highly prevalent in the human population, is the main etiological agent of genital ulcers and neonatal encephalitis and produces significant morbidity. Noteworthy, HSV-2 interferes with the maturation and viability of dendritic cells (DCs), key immune cells involved in mounting antiviral responses to limit disease and virus spread, thus likely affecting the capacity of the host to mount effective adaptive immune responses against this virus. Recently, we described an HSV-2 virus that is deleted in *US6* gene (encoding glycoprotein D, gD). This virus can be propagated on a complementing cell line that encodes gD from HSV-1, giving rise to a single-round replication virus named ΔgD-2. Importantly, this mutant virus was reported to be safe, immunogenic and induce protective immunity against challenges with high doses of clinical isolates of HSV-1 and HSV-2 in mice. Here, we show that ΔgD-2 is attenuated in DCs unlike wild-type HSV-2 (WT) and other HSV-2 glycoprotein-mutants. In addition, ΔgD-2 induces DC maturation and promotes antigen processing, whereas the opposite was observed after inoculation with the WT virus. We found that DCs exposed to the WT and ΔgD-2 viruses experienced different unfolded protein responses. Furthermore, DCs inoculated with ΔgD-2 activated virus-specific $CD8^+$-T cells (gBT-I) and antigen-specific $CD4^+$-T-cells (OT-II), *in vitro*. Finally, mice inoculated with ΔgD-2 displayed increased migration of $CD103^+$ DCs to lymph nodes, promoted DC maturation *in vivo* and activated both, $CD8^+$ and $CD4^+$ T cells. Taken together, our findings suggest that ΔgD-2 activates DCs and promotes antigen presentation to T cells, which likely contributes to the induction of protective immunity observed.

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Salmonella Typhimurium virulence factors SopB, SptP and PphB contribute to subvert autophagy in _Dictyostelium discoideum_ and RAW264.7 macrophages

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Autophagy plays a crucial role during infection acting as a defense mechanism against intracellular pathogens. As a consequence, many bacterial pathogens have developed the ability to evade this process. Autophagy is regulated by several intracellular signaling pathways, such as those linked to MAPK and PI3K/AKT/mTORC1. In these pathways, activation of MAPK and/or mTORC1 results in the repression of autophagy. In this work, we evaluated the contribution of _S_. Typhimurium virulence factors SopB, SptP and PphB in the evasion of autophagy in _Dictyostelium discoideum_ and RAW264.7 macrophages. SopB is a phosphatidylinositol phosphatase that activates AKT, SptP has a tyrosine phosphatase domain that inactivates Erk, and PphB is a non-characterized hypothetical serine-threonine phosphatase. We constructed Δ_sopB_, Δ_sptP_ and Δ_pphB_ mutant strains derived from _S_. Typhimurium 14028s expressing the red fluorescent reporter mCherry, and performed infection assays using _D. discoideum_ GFP-Atg8 and RAW264.7 EGFP-Atg8 macrophages at a multiplicity of infection of 1,000 bacteria/cell. After 45 min of infection, the number of autophagosomes was determined in _D. discoideum_ and RAW264.7 macrophages by confocal microscopy. The analysis of our results indicate that SopB and PphB inhibit the generation of autophagosomes, while SptP induces the generation of autophagosomes during _S_. Typhimurium infection of both host cells. In conclusion, the virulence factors SopB, SptP and PphB of _S_. Typhimurium contribute to subvert autophagy in the amoeba _D. discoideum_ and RAW264.7 macrophages.

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ORAL SESSION III

Chairs: Dr. Juan Carlos Salazar and Dr. Cecilia Toro

Primary production and inorganic nitrogen uptake by active microbial community in the central Arctic Ocean (4-89.5°S)

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This research was conducted in the Amudsen Basin close to North Pole during the boreal summer 2012 (LOMROG III expedition), and was focuses on N and C uptake by active microbial communities living in ice-brine and seawater, whose identity was reveals by metatranscriptomic analyses. The most active members of microbial community were Bacteroidetes (Flavobacteria), and Proteobacteria (α-, ß- and γ-) as well as Alveolates (Ciliophora and Dinophyceae), Stramenopiles (Bacillariophyta) and Haptophytes (Phaeocystales) at both ice-brine and seawater.Nitrate uptake rates fluctuated from 0.95 to 165 nmol L^{-1} d^{-1}, being one order of magnitude higher under light (30.2±9.86 nmol L^{-1} d^{-1}) than at darkness condition (3.69±1.26 nmol L^{-1} d^{-1}), reaching 52.1% during light conditions in seawater. Also, NO_3^- uptake correlated positively to *nar*B gene transcripts affiliated to Corynebacteriales, Burkholderiales, Rhizobiales and Flavobacteriales. In contrast, ammonium uptake rates were 5 times lower than nitrate, ranging between 1.01 and 21.8 nmol L^{-1} d^{-1} (mean±SD: 6.69±2.49) and showing not differences between both habitats and number of *gln*A gene transcripts. Inorganic C-assimilation fluctuated from 89.1 (SD±16) to ice-brine and 399 nmol L^{-1} d^{-1} (SD±92) to seawater, where approx. 82% of this assimilation was due to photosynthetic eukaryotes revealed by high transcripts of *psb*A/*psa*A and *rbc*L genes. Finally, Bacteroidetes/Proteobacteria and Stamenopiles/Haptophyte were the most dominant and active phyla supporting the new/regenerated and primary production in Arctic Ocean. Therefore, changes on microbial communities, point out the relevance of understanding the effect of rapid environmental changes (freshening and warming) in the Pole.

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Diversity of adhered microbial communities in colon mucosa biopsies from Chilean and Spanish patients with ulcerative colitis or Crohn's disease

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Background. Dysbiosis has been associated with changes in the individuals diet, antibiotic use and several clinical disorders, including inflammatory bowel diseases (IBDs). Identification of the microbiota adhered to the intestinal mucosa has been made mainly using short sequences (<300 nucleotides) difficulting accurate affiliation at the genus or species level. The aim of this study was to characterize the microbiota of colonic mucosa obtained from Chilean, Spanish IBD patients and control subjects, using high quality and longer reads (>300 nucleotides) for affiliation process. **Methods.** 16S rRNA gene partial sequences were amplified from biopsies taken from 20 patients with Ulcerative colitis (UC), 21 patients with Crohn's disease (CD) patients and 9 healthy subjects (CTL) from Chile, by 454 GS FLX Plus System. The phylogenetic inferences from reads were grouped in OPU through ARB software. **Results**. Chilean and Spanish patients with CD and Chileans with UC may be differentiated into 4 groups based on the composition of their gut microbiota. Among these, groups IBD-2 and IBD-3 showed predominance in the proportion and abundance of *Proteobacteria* (57% and 52%, respectively), while the most frequent taxon in the IBD-1 group was *Bacteroidetes* (34.5%). The dominant species of *Proteobacteria* in IBD-2 were OPU-28 *Cupriavidus necator/Ralstonia pickettii.* In IBD-1 and IBD-3, the dominant species were OPU-86 *Bacteroides dorei/Bacteroides vulgatus* and OPU-484 *Lactobacillus acidophilus*, respectively. On the other hand, control samples were dominantly classified into groups IBD-4/CTL. Group IBD-4/CTL had a larger proportion of the phylum *Firmicutes* (60%), with predominance of *Faecalibacterium prausnitzii* (14.59%), while IBD-5/CTL showed an increase in *Proteobacteria* (69%), with predominance of *Klebsiella oxytoca*. **Conclusion.** We could not differentiate patients with UC from those with CD by the microbial composition of their intestinal microbiota. Nevertheless, IBD patients may be classified into 3 groups according to the type of dysbiosis observed; these three groups are distinguished by an increase in the phyla *Proteobacteria* and *Bacteroidetes* and a decrease in *Firmicutes*. The abundance of OPUs from these phyla, along with a decrease in OPUs of the class *Clostridia*, such as *Faecalibacterium prausnitzii* and *Eubacterium rectale,* is characteristic of individuals with IBD.

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Identity and expression of Proteorhodopsin genes in Chile Bay, Antarctica

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Proteorhodopsin (PR)-based strategy utilizes a single integral membrane protein covalently bonded to retinal, which function as a light-promoted proton pump. PR-containing bacteria are assumed to use both light and organic compounds as energy sources. However, photoheterotrophic strategies are not yet considered in many models of organic carbon flow, although photoheterotrophic bacteria that use PR represent from 15 to 70% of the total prokaryotes in surface waters of different oceans. To date, there is genetic evidence of the abundance of this gene in marine systems, and the physiological importance of this protein, based on experiments in marine isolates that present PR. However, these evidences are limited to tropical waters and cold systems such as the North Atlantic and the Arctic Oceans, but no evidence has been reported for PR in Antarctic marine systems. Through metagenomic and metatranscriptomic analysis we have determined the identity of microorganisms that present PR and the transcriptional activity of this gene in coastal Antarctic waters of Chile Bay (Greenwich Island) during the austral summer. Our results demonstrate that in Chile Bay waters, there is a prokaryotic community that presents PR and this is actively transcribed during day and night time. Those PR gene sequences are mainly affiliated to Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes. Bacteroidetes PR gene sequences are the most abundant and active in the metagenomes (~70-90%) and metatranscriptomes (~60%) respectively, despite that the most abundant Phylum in Chile Bay at summer is the Proteobacteria (80% of assigned reads).The discovery of PR in Antarctic coastal waters represents the first genetic evidence of PR-based photoheterotrophy in these extreme marine region. This has a significant impact on conceptual models for this marine region, demonstrating a new strategy by which the energy of light could be used by the Antarctic biosphere.

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Centro de Ciencia del Clima y la Resiliencia (CR)2: Project FONDAP 15110009

Metabolic profiles of bacteria associated to Antarctic sponges indicated by whole-genome analysis

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Sponge microbiome is composed of a wide range of microbial communities which display a key role in different aspects of the sponge biology and in biogeochemical cycling of key elements for ecosystem functioning. Studies about the metabolic role of microorganisms isolated from Antarctic sponges have been mainly focused on the analysis of bioactive molecules production with pharmaceutical or industrial interest. Here, we studied the metabolic potential of nine bacterial strains isolated from two Antarctic sponges, with a special emphasis on nutrient cycling and symbiotic life style, using Illumina sequencing. Here, we performed whole-genome sequencing and genomic comparative approaches to access the metabolic potential and symbiotic features. Taxonomic analysis was performed based 16S rRNA gene Sanger sequencing of each isolate. Bacteria isolated were assigned to the genera *Pseudoalteromonas* (E16.1, E19.1, 19.4), *Nesterenkonia* (E16.7, E16.10), *Sporosarcina* (E16.8), and *Cellullophaga* (E16.2). Using high-throughput sequencing data, contigs >200 bp were used to perform ORFs prediction, and functional annotation using Prokka (Seemann 2014). CRISPR system was predicted by CRISPRFinder (Grissa, Vergnaud, and Pourcel 2008). Number of sequenced reads, in average, was 2.416.522. Genomic completeness of each isolate was estimated as higher to 98% for all genomes. Bacterial strains isolated display metabolic potential to perform: i) nitrite reduction (Nitrite reductase, *nir* gene; E16.1, E16.7, E16.10, E19.1, E19.4) ii) nitrate reduction (nitrate reductase, *nap* gene; E16.1, E16.2, E19.1, E19.4), iii) glycolysis (e.g. glucose-6-phosphate isomerase, *pgi* gene; all samples) and iv) phosphorous cycle (i.e. Polyphosphate kinase, *ppk* gene; E16.2, E19.3 and E19.4). Interestingly, Cellulophaga has genomic elements associated to CRISPR systems and CRISPR-associated proteins Cas1, Cas2, Cas9, and Eukaryotic-like (e.g Tetratricopeptide repeat), suggesting that this bacterial isolate could be a sponge symbiont or sponge associated bacteria. Overall, genomic features in bacterial strains isolated from Antarctic sponges shown a metabolic potential related to nutrient cycling and display several signatures related to symbiotic lifestyle, extending previous results from tropic and temperate environments to polar systems.

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Isolation and identification of endophytic fungi from two poaceae plant species growing on a mine tailing facility in southern Chile

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Endophytic fungi could improve host plant growth in polluted soils with heavy metals. Three endophytes fungi were isolated from two plant species, *Poa pratensis* and *Poa stuckertii* that grow spontaneously on a mine tailing facility in Southern Chile. In this work, we identified the three fungi isolates using ITS1/ITS4 markers and morphological characteristics observed in aerial hyphae from slide culture. The endophytic fungi belong to the Ascomycota Division, two of the isolates were assigned to the Xylariales and one to the Hypocreales Order. These fungal endophytes might be interesting in bio-assisted phytoremediation.

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Comparing microbiota between wild and reared fish: exploring microbial clues for aquaculture diversification

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Aquaculture has become one of the fastest growing animal production sectors in the world. In this scenario, Chile has an interesting opportunity for diversification because of its experience in intensive farming (salmonids) and long coastal area, which houses several native species. One of the main challenges for diversification in aquaculture facilities is the control of diseases, particularly during early life stages. Considering the key role of microbiota on healthy status of the host, we use next generation sequencing technology to determine the microbiota composition in fish of commercial interest belonging to the following orders: Pleuronectiformes (flounder, *Paralichthys adspersus*), Perciformes (yellowtail, *Seriola lalandi*) and Ophidiiformes (conger, *Genypterus chilensis*). Knowledge of microbiota may help to improve the cultivation of thoses species; however, few comparative studies have evaluated the intestinal microbiota composition in farmed versus wild fishes. The comparison between wild and aquaculture specimens revealed important differences in the composition of the microbiota (ANOSIM and PERMANOVA). Wild flounder showed a predominance of Proteobacteria (70%), while aquaculture flounder were dominated by Firmicutes (60%). Specific taxa were found to be differentially distributed between aquaculture and wild flounder; *Bacillus* and *Pseudomonas* were highly represented in aquaculture, meanwhile, *Arthrobacter* and *Psychrobacter* were observed in wild flounders. In yellowtail, a similar tendency in phylum distribution was observed, but a total of thirteen genera were differentially represented between the two origins. In conger, Firmicutes were dominant in reared fish, but Tenericutes were dominant in wild fish. Contrarily, in salmonids, Proteobacteria were dominant in reared fish, but Planctomycetes and Firmicutes were dominant in wild fish. These results are interesting in the context of co-evolution between the host and its microbiota because co-evolution is believed to have been an important mechanism in the formation of the host-gut microbe relationship. However, factors such as diet may influence the composition of the gut microbiota and consequently the metabolic functions of intestinal microbes, with effects on the host's nutrition and immune defense.

FONDECYT 1171129

ORAL SESSION IV

Chairs: Dr. Fernando Valiente-Echeverría and Dr. Luis Castillo

Identification of bacteria with probiotic activity against *Flavobacterium psychrophilum* from the microbiota of the gastrointestinal tract of *Salmo salar*

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One of the main problems facing the national salmon industry is the spread of outbreaks of pathogens of both bacterial and viral, causing great losses. To control these pathogens, fish are mainly treated with antibiotics and vaccines in addition to other prophylactic measures.Being antibiotics one of the most used measures, however its excessive use it has negative repercussions for public health, the environment and the economy. In addition to not being selective in their action inhibiting both pathogenic and commensal microorganisms that make up the microbiota of the gastrointestinal tract (TG) and mucous membranes of the fish, which acts as a barrier to protection against potential pathogens. The main bacterial pathogen that affects the production of salmonids in freshwater culture centers is Flavobacterium psychrophilum, responsible the loss of up to 70% of the fry population, In spite of the importance of this pathogen in the cultivation of salmonids, there is no effective method for preventing infection. Based on this background we propose as hypothesis that "The *Salmo salar* microbiota presents microorganisms with probiotic activity that can be used to protect the fish against the action of *Flavobacterium psychrophilum*". For what was proposed as methodology: characterize the total microbiota of *Salmo salar* of samples of TG y gills by means of metagenomic analyze, based on 16S rRNA. The identified genera or species were then isolated. For the isolation culture media are used that promote the growth of these microorganisms. Once the colonies were obtained, they were identified by means of analysis of the 16S rRNA gene. Subsequently, the inherent antibacterial capacity of these microorganisms against *Flavobacterium psychrophilum* was evaluated by microbial antagonism assays. Our results allowed the identification of 61 colonies with different morphology from TG samples, which were exposed to *F. psychrophilum*, generating inhibition halos up to 20 mm. It is possible to conclude that in TG *Salmo salar*, there is the presence of bacteria that could be used in the generation of probiotics for the salmon industry.

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The ratio of phosphoenol pyruvate/pyruvate modulates the stability of *Escherichia coli* and *Bacillus subtillis* FtsZ polymers

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Bacterial division is a key process with at least a dozen of proteins involved, among them FtsZ and FtsA or ZipA, that form the Z-ring in the middle of the cell. Z-ring is the scaffold that recruits the rest of the proteins to form a complex called the *divisome*. Key for divisome formation at the division site is FtsZ, a GTPase that responds to several modulatory inputs. In the two best studied bacteria, *E. coli* and *B. subtilis*, cell division is regulated at the level of FtsZ assembly because no significant changes seem to occur in the concentration of FtsZ, either throughout the cell cycle or under different growth conditions. A regulation between nutrition and bacterial growth and division, has been proposed in *B. subtilis*, where metabolic sensor detects the nutrient status and transmits this information to the division machinery. According with this mechanism, we propose that the ratio of phosphoenol pyruvate (PEP)/pyruvate (Pyr), could be involved in the modulation of Z-ring formation. Specifically, our in vitro and crowding experiments of light scattering, electron microscopy and GTP hydrolysis showed that the presence of PEP (cyclohexilammonium salt), in the range of intracellular concentrations, enhances polymerization and increases the stability of the polymers of *E. coli* and *B. subtilis* FtsZ. In vitro the effect of PEP was specific because acetyl phosphate (Li^+-K^+ salt), 2-phosphoglycerate (Na^+ salt) and pyruvate (Na^+ salt) had no effect. However, in crowding conditions pyruvate diminished the stability of the polymers induced in the presence of PEP. The results as a whole suggest that FtsZ polymerization could be modulated by the [PEP]/[Pyr] ratio in the micromolar range. This finding is of great interest, as there is little knowledge of regulation by intermediate glycolytic metabolites on cell division that could be related with nutrient availability.

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Novel antifungal peptide secreted by candida intermedia lamap1790 active against *Brettanomyces bruxellensis*, a wine spoilage yeast

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Brettanomyces bruxellensis has been described as a principal contaminant of the wine industry. To avoid its growth, the must is commonly supplemented with SO_2, one of the most used preservatives in the food industry. However, the use of this compound has been questioned for the potential health problems that entails its consumption. With the aim of exploring other alternatives, our laboratory has previously described a strain *Candida intermedia* LAMAP1790 as a producer of antimicrobial compounds. Thus, the objective of this work was to determine the antifungal activity of the compound produced by *C. intermedia* against *B. bruxellensis* and elucidate its chemical nature. Using joint seeding tests and viability after direct exposure to sterile culture medium of *C. intermedia* LAMAP1790, the secreted compound was found to have fungicidal activity on four strains of *B. bruxellensis*, without affecting the growth of *Saccharomyces cerevisiae*. Posteriorly, in order to determine the chemical nature of the compound, the proteins in the supernatant were fractionated and concentrated 250X, which revealed that the antifungal activity is related to the presence of two peptides with a molecular mass less than 5 kDa. Using 2D-nano-LC-MS/MS we reported the sequence of a 33 residues cationic peptide present in the culture media, whose molecular mass was 3.2 kDa and an isoelectric point of 9.72. Finally, using a bioinformatics approaches, it was determined that the peptide present homology to a transmembrane segment of yeast MIP aquaporine superfamily and the 3D structure corresponds to a positively charged amino terminal segment followed by an α-hélix structure that stabilize the hydrophobic residues, allowed its amphiphilicity. These results allow to establish that the secreted peptide have biochemical and structural similarities to antimicrobial peptides (AMPs) as the ones described in filamentous fungi, with possible biotechnological applications in the control of spoilage microorganisms in the wine industry.

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Origins and evolution of the thermal cyanobacteria *Mastigocladus* sp.: lights from comparative genomics

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Cyanobacteria are cosmopolitan photosynthetic organisms with a great morphological diversity. The order Stigonematales comprises filamentous true-branched trichomes with the capacity to differentiate heterocysts. In addition, some of its members are dominant in microbial mats with temperatures between 40 and 60°C distributed around the world. In these systems, it has been shown that the greater part of the carbon fixed by photosynthesis and the de novo nitrogen input is due mainly by the populations of the genus called *Mastigocladus* (which includes thermal strains of the genus *Fischerella*). Through phylogenetic and phylogenomic analysis, we compared Stigonematales genomes from public databases (17), sequenced in our laboratory (2) and binned pangenomes obtained from metagenomes of Porcelana hot spring (3). Our results demonstrate that *Mastigocladus* genus (thermal genomes) is monophyletic and its evolutionary history suggests that this unique event of ecological specialization to hot springs, came from an ancestor of the genus *Fischerella* from freshwater or terrestrial environments. Thermal genomes are smaller, about ~ 6 Mpb compared to mesophilic ancestors (> 7 Mpb); and we saw a reduction in some pathways like secondary metabolite synthesis. From the comparative genomics, we determined that there is a genomic core of approximately 3000 CDS shared by these two groups, while each of them presents more than 5% of exclusive genes. Furthermore, other monophyletic groups of cyanobacteria usually are present in microbial mats from hot springs (Oscillatoriales and Thermosynechococcales) and together *Mastigocladus*, we found a set of 13 CDS shared and with hight identity among all these phylogenetically unrelated thermophilic cyanobacteria. By phylogeny, we also observed that these correspond to horizontal transfer events (HGT) from other phyla and that three of them increase in abundance at higher temperatures in our metatranscriptomic data from Porcelana. These genes could represent a unique evolutionary model associated with the ability to live at high temperatures acquired by these cyanobacteria that today inhabit thermal systems around the world and that could have biotechnological potential.

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Effect of salinity on microbial activity and bacterial diversity in a wastewater biofilter using volcanic stones as filter bed

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Although treated wastewater reuse is recognized as a strategic option in augmenting agricultural water supplies in arid and semi-arid areas, there are many challenges that face the utilization. Social and economic considerations should be considered in developing reuse options and strategies. In order to increase the hydric sustainability of rural communities of the Chilean Altiplano, we designed and implemented a laboratory scale biofilter system with volcanic stones, and the goal of this work was to determine the effect of wastewater salinity on microbial activity and bacterial diversity. The biofilter was constructed with an acrylic tube (0.14 m inner diameter) which was filled with volcanic stones from Toconao. The system was inoculated with activated sludges, and the chemistry oxygen demand (COD) of synthetic water reached average value of 120 mg/L. The conductivity tested values were 1.73, 3.1, 5.2, and 8.8 ms/cm, and residence time of 7 h and flow rate of 0.2 mL/seg were used. The results show that the efficiency of organic matter removal was significatively affected in the higher salinity (efficiency of 60 %). To determine the microbial activity associated to volcanic stones biofilms, acid phosphatase and alkaline phosphatase activities were measured. The activities showed average values of 0.15 mM/min x gSSV and 0.037 mM/min x gSSV, respectively. The bacterial diversity in stone biofilms was determined by using massive sequencing of 16S rRNA genes. In low salt concentration samples (1.73 and 3.1 ms/cm), the bacterial communities were dominated by *Proteobacteria* (56.6-46.6 %) followed by *Bacteroidetes* (18.5 and 13.8 %). Among *Proteobacteria*, the proportion of *Alphaproteobacteria* (30.1-24.1 %) was much higher than *Gammaproteobacteria* (10.2-6.1 %). In high salt concentration samples (5.2 and 8.8 ms/cm), the bacterial communities were dominated by *Proteobacteria* (42 %) followed by *Bacteroidetes* (30 %). The abundance of *Gammaproteobacteria* class increased to 24 %, and the most frequent family within this group was *Chromatiaceae* (18 %). *Flavobacteriaceae* (17 %) was the major family group within the *Bacteroidetes*. The efficiency of organic matter removal and the microbial activity were inhibited by higher salinity, and consequently changes in bacterial diversity were observed in these operation conditions.

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Characterization of lytic bacteriophages and determination of theireffectiveness for the biological control of important pathovars of *Pseudomonas syringae*

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Plant diseases caused by *Pseudomonas syringae* are a worldwide agricultural problem, due to its persistence and ability to infect a large variety of plant hosts, generating big economic losses. The main control mechanisms used to prevent the infection is by controlling the good agronomic practices that prevent its spread, and the use of inorganic copper compounds and antibiotics. However, the permanent and excessive exposure to these compounds has generated phytotoxicity and the emergence of resistant bacterial strains. Currently, the use of biocontrollers that have a neutral or positive environmental impact, such as lytic bacteriophages against the bacterial pathogen, is an innovative and effective strategy. These have the advantage of a rapid spread and high host specificity. Therefore, our aim is to isolate and characterize bacteriophages with bactericidal activity against *Pseudomonas syringae* and to determine their effectiveness as biocontrollers of different pathovars of this bacterial specie. To achieve this goal, we are working in the bacteriophages isolation with activity against *P. syringae*, in the characterization of their genomes, in the determination of virions protein composition and analysis of ultrastructure of the bacteriophages.–In our laboratory, at least 3 bacteriophages have been isolated and their characterization is ongoing. Their ability to infect and destroy by cellular lysis to different pathovars of the *P. syringae* bacterium has been successfully evaluated, by inhibition of growth of *P. syringae* in solid and liquid medium, and by protection of tomato plants against the bacterial infection. According to data obtained by electron microscopy were determined to have capsids with icosahedral geometry and short tails. During infection, viral particles were detected on the surface and cytoplasm of bacteria. In addition, they were determined to possess double-stranded DNA with an average size of approximately 35-40 kbp. According to our results, the isolated bacteriophages could be belong to the *Podoviridae* family and to the Caudovirales order. Finally, because of its ability to lyse a group of pathovars of *P. syringae*, they are promising candidates to generate a protective formulation against the infection.

Universidad de Santiago de Chile

POSTER SESSION I

CLP1-01

Environmental resistomas in thermal waters of the Urauco Spa. Province Pichincha. Ecuador

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Ecuador is within the circle of the Pacific, this geographic location allows the groundwater circulating in the soil to acquire a high geothermal degree, manifested by the surge of hot waters linked to volcanic rocks. In these waters, there is an autochthonous bacterial population that has not yet been characterized and it is not known if they have been exposed to contaminants such as antibiotics. To carry out a study of the antibiotic resistance profiles of the bacteria present in the thermal waters of the Urauco Spa in the Lloa, belonging to the city of Quito, Pichincha Province, Ecuador, an altitude of 2773 m.a.s.l. Isolation of bacterial species was performed by culturing the samples in the Petrifilm medium (3M) for heterotrophic bacteria. Taxonomic identification was carried out using biochemical tests as indicated by MacFaddin (2003) and supplemented by tests contained in the API galleries (BioMeriaux). Susceptibility to antibiotics was determined by the Kirby-Bauer method. *Acidovorax, Aeromonas, Pasteurella, Citrobacter, Brevundimonas, Flavobacterium, Vibrio, Budvicia* and *Bacillus* were isolated and identified. It was shown that *Aeromonas salmonicidas subsp. Salmonicide*, *Pasteurella multocida*, *Citrobacter freundii* and *Aeromonas caviae* have multiresistance to antibiotics: ampicillin, amoxicillin/ Clavulanic acid and Cephalothin, whereas *Bacillus* spp. was multiresistant to ampicillin, penicillin and oxacillin. In conclusion, great diversity and a considerable amount of resistant and multiresistant bacteria were found to several antibiotics, evidencing the existence of environmental resistomas in these aquatic ecosystems and possible contamination of these waters with antibiotics.

ESPOCH. UCE. ULA

CLP1-02

Metabolomics-guided identification of a cyclic peptide with antimicrobial activity from a Chilean marine actinomycete

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Actinomycetes have been widely recognized for the capability to produce a myriad of secondary metabolites presenting various bioactivities. The marine environment provides an excellent alternative to discover novel bioactive compounds, as the bioprospection of undescribed marine-derived actinomycetes may lead to unveil novel natural products. In this context, the exploration of the marine *Streptomyces* sp. H-KF8 obtained from a remote fjord located in Northern Chilean Patagonia was proposed. Antimicrobial activity of this fjord-derived strain, demonstrated an important antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*, showing a promising potential to produce bioactive compounds. Therefore, its whole genome was sequenced, representing the first Chilean marine actinomycete genome reported so far, with a 7.6 Mbp genome assembled into 11 contigs, containing 6,486 coding sequences. Genome mining of *Streptomyces* sp. H-KF8 led to the identification of 26 biosynthetic gene clusters (BGCs) for secondary metabolites, including 2 polyketide synthases (PKS), 2 non-ribosomal peptides synthetases (NRPS) and 5 hybrid PKS-NRPS. Of all BGCs, 81 % presented low similarities with known-BGCs, suggesting that it possess novel metabolic routes that may be involved in the synthesis of novel compounds. Organic solvent extractions from liquid cultures of *Streptomyces* sp. H-KF8 and HPLC- bioguided fractionation of the crude extract were performed. Mass spectrometry (MS) techniques such as ESI-FT ICR, MALDI-TOF and Imaging MS were used to detect molecules related to antagonistic bacteria interactions, revealing three masses that presented a sustained correlation with the monomer composition predicted by genome mining of one NRPS cluster. This cluster is involved in the synthesis of a cyclic peptide core composed of D-amino acids and non-proteinogenic ones, with additional functional groups such as sugar moieties added by tailouring reactions. Altogether, these results suggest that Chilean Patagonian fjords are suitable environments for bioprospecting actinomycetes with promising potential in natural product discovery.

CLP1-03

In the search of a common mechanism of toxicity and response to the soft-metal(loid)s Ag(I), As(III), Au(III), Cd(II), Hg(II) and Te(IV) in *Escherichia coli*

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Background. Our laboratory has been interested for many years in the toxicity and bacterial resistance to the metalloid tellurium (Te) which is a non-essential metal(loid) as Hg, Au, Ag, As and Cd, among others. This kind of metal(loid)s are extremely toxic even at low concentrations and do not exhibit a biological role to date. Interestingly, an important number of these metal(loid)s can be classified as soft-metal(loid)s because they share some chemical properties as inter alia, thiol reactivity and high polarizability. In bacteria, soft-metal(loid)s like Hg, Ag, Au, Cd, As and Te provoke ROS-mediated cellular damage, direct damage to proteins containing iron-sulfur clusters and/or exchange of catalytic metals. Because of the similarity in their cell targets and chemical properties our aim is to understand how *E. coli* responds to these metal(loid)s and what are the targets of these metal(loid)s in ROS-free conditions (anaerobiosis). **Methods.** We performed a chemical genomics analysis, which is basically a genome wide approach consisting of a pool of mutants in all the non-essential *Escherichia coli* genes. The entire pool was independently exposed to the soft-metal(loid)s Ag, Au, As, Cd, Hg and Te. The abundance of each mutant was quantified by Illumina sequencing and the Chemical Genomic score (CG) was calculated. Enrichment analysis (GSEA), hierarchical clustering (WebGimm) and Venn's diagrams (jvenn) were carried out to find out common cellular targets and common cell responses to these metal(loid)s. **Results.** The chemical genomics profiling showed that mutations in some pathways, biological functions and transcription factors affect the fitness of *E. coli* challenged with soft-metal(loid)s. Venn's diagrams, hierarchical clustering and enrichment analysis revealed genes involved in LPS and peptidoglycan biosynthesis, cell division, DNA repair by homologous recombination and transcription regulation of copper-detoxifying systems are required for resistance to all the soft-metal(loid)s tested in this study. **Conclusions.** In this study, we address an unaddressed question about the toxicity and the response to soft-metal(loid)s in *E. coli*. We demonstrate that apparently they share some common cellular targets (DNA, peptidoglycan and cell division) and the response to them is maybe controlled by copper responsive transcription factors.

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CLP1-04

Antimicrobial resistance of Shiga toxin-producing *Escherichia coli* (STEC) isolated in Chile

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Background. Shiga toxin-producing *Escherichia coli* (STEC) is an emerging pathogen that causes foodborne outbreaks. Human disease range from mild, self-limited diarrhea to life threatening complications such as the hemolytic uremic syndrome. The bacteria represent a serious public health concern; therefore, the study of STEC antimicrobial resistance (AMR) is relevant. STEC AMR has been described previously in the world and in Chile, but recent information is missing. The aim of this study was to determine AMR in STEC isolated in Chile from different origins. **Methods.** We studied 99 STEC isolated from ground beef (40), cattle (53), wild birds (2), cheese (3) and a clinical isolate (1). Isolates were previously confirmed as STEC, and 17 carried gene *stx1* (17.8 %), 72 harbored *stx2* (72.7%), and 10 isolates carried both Shigatoxin genes (10,1%). We determined *in-vitro* antimicrobial resistance to ampicillin (AMP), amoxicillin/clavulanic acid (AUX), ceftiofur (TIO), ceftriaxone (AXO), cefoxitin (FOX), imipenem (IPM), gentamycin (GEN), streptomycin (STR), azithromycin (AZI), tetracycline (TET), ciprofloxacin (CIP), nalidixic acid (NAN), sulfisoxazole (FIS), trimethoprim/sulfamethoxazole (COT) and chloramphenicol (CHL) through disc diffusion technique. The Clinical and Laboratory Standards Institute (CLSI) breakpoints were used for interpretation of the inhibition halos. **Results.** We found that 7% (7) of STEC isolates presented antimicrobial resistance or decreased susceptibility to STR, FIS, COT, TET, AMP and/or NAN. Interestingly, all resistant strains were resistant to TET. Strains were from ground beef samples (3), cattle (3), and a clinical sample. All three ground beef samples were multiresistant and displayed the resistance profile TET-FIS and STR. One of the cattle samples was resistant to TET, and the remaining two were has a reduced antimicrobial susceptibility to NAN and AMP. Finally, the clinical isolate was resistant to 5 antimicrobials: FIS-STR-AMP-COT-TET. **Conclusion.** STEC isolated from different origin display antimicrobial resistance to different antimicrobials. Consumers could be in direct contact with multi-resistant STEC through manipulation of ground beef.

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CLP1-05

Effect of cadmium nanoparticles (quantum dots) against human flagellate protozoan parasites

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Human infections by flagellate protozoan parasites constitute a public health problem at the global level. Among them, *Trypanosoma cruzi* infects 7-8 million people around the world and, *Trichomonas vaginalis* infects 270 million people every year. In the case of *T.cruz*i infection, only two drugs are available for treatment, which are active mainly in the acute phase of the infection and have multiple side effects. In the case of *T. vaginales* infections, the treatment is made with 5 nitroimidazolic, derivatives such as Metronidazole, against which 5-10% of clinical origin strains present resistance. Then in both infections, new therapeutic alternatives are necessaries. In recent years, nanoparticles (NPs) have emerged as new chemotherapeutics options that have been explored to pathogens. However, its activity has not been reported against *T. cruzi* and *T. vaginalis.* Then, the main goal of this works was to evaluate the protozoicidal activity of cadmium NPs (CdNPs). The CdNPs activity against *T. cruzi* was performed using epimastigotes expressing β-galactosidase. Two-fold dilution of each NP were performed and the assays were incubated by 48 h at 37 °C. Then, the red phenol β-D galactopyranoside (CPRG) was added as a substrate of β-galactosidase and the absorbances were evaluated at 570 nm. On the other hand, *T. vaginalis* trophozoites were incubated at 37 °C in the presence of two-fold dilutions of each CdNP, as described above. The trichomonicidal effect was evaluated by microscopic counting in the Neubauer chamber and the percentages of mortality were determined with respect to the controls incubated in the absence of CdNPs. All CdNPs were actives against *T.cruzi*. The study of LD_{50} showed that this was 91.2μg/mL^{-1}, for CdNP 2; 295.12 μg/$mL^{-1}$for CdNP3 and 118.03 μg/mL^{-1}, for CdNP 4. The trichomonicidal activity of CdNPs shown that LD_{50} of CdNP 2 and 3 was 62.5 μg mL^{-1}. In the same way, LD_{50}, of CdNP 4, was 125μg/mL^{-1}. Then, we can conclude that CdNPs can represent a good chemotherapeutic option against human infections by flagellate protozoan parasites.

CLP1-06

Characterization of genetic elements involved in multiple antibiotics resistance in a florfenicol-resistant bacterial bank made from cultivable bacteria associated to salmon farming in Chile

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The antibiotic overuse is a common feature in human and veterinary health. As a consequence of this practice huge amounts of antibiotics has been released in the environment. Chilean salmon industry is a good example of this constant pollution with antibiotics. Since 2007 more than 4000 tons of antibiotics have been used to control bacterial diseases in salmon cultivable species. The main problem of the misuse of these drugs is the selection of antibiotic resistance bacteria not only the pathogens, because the most affected microorganisms are the environmental bacteria. Florfenicol and oxytetracycline are the principal antibiotics in the Chilean salmon farming. In order to unravel and describe the impact of the excessive release over different bacterial populations we isolate resistant bacteria from the marine sediments, the water column and the fish intestinal microbiota of *Salmo salar*. With these bacteria a florfenicol resistant bank was constructed. Samples were obtained from three farms with a historical high antibiotic use and as a control a fish farm with low antibiotic. In this bank just isolates with a CMI > 128 µg/mL were included. The multi-antibiotic-resistance is an extended phenomenon in environmental and bacterial pathogens. In this work the florfenicol-resistant bank was challenged with different antibiotics like chloramphenicol, ampicillin, tetracycline, erythromycin and to a lesser extent kanamycin and ciprofloxacin. In at least 70% of the bank members the cross-resistant phenomenon is common. In the same way a complete screening looking for the common resistance genes against florfenicol was performed. A high incidence of *floR* and *fexA* genes was found. Both genes code for Major Facilitator Superfamily efflux pumps (MFS) responsible to resistance to florfenicol and chloramphenicol. On the other hand, the presence of tetracycline, macrolide and class 1 integrase resistance genes was found, fact which could even drive the mobilization of multiple resistance elements and the spread to susceptible bacteria in the natural ecosystems.

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CLP1-07

Characterizing trimethoprim resistance genetic elements in Chilean strains of *Shigella sonnei*

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Shigella sonnei is one of the most frequent etiologic agents of shigellosis, a foodborne diseases in industrialized countries. In Chile, *S*. *sonnei* is regularly isolated and most of the strains are resistant to Trimethoprim (TMP^R). Previous molecular characterization of TMP-resistance genes in Chilean *S. sonnei* identified *dfrA1*, *dfrA8*, *dfrA14* and *dfrA15* alleles, differentially distributed along the time. The presence of *dfrA8* was detected in strains isolated before the 2009-outbreak, and *dfrA14* mostly in SRL-positive multiresistant strains belonged to the 2009-outbreak. After that (2010-2013), besides *dfrA14*, *dfrA1* and *dfrA15* were detected mostly in strains positive for class 2 integron. To characterize the genetic context of *dfrA15* in 212 chilean *S. sonnei* strains isolated between 1995-2013 (28 of them TMP-susceptible), conventional and tiling PCR were used to detect class 2 integron genetic organization. Clonality was analyzed by PFGE. Results showed that *dfrA1-dfrA15* combination was distinguished in two different clones. Especifically, one including TMP^R nalidixic acid-resistant strains with atypical Class 2 integron (*dfrA1-sat* cassette array). The other one, TMP^R nalidixic acid-sensitive strains linked to a typical Class 2 integron (*dfrA1-sat-aad1* array). This distribution was detected in strains isolated after the outbreak. Thus, the presence of different TMP-resistance markers allows detecting variants of *S. sonnei* strains highlighting a dynamics in the circulating population.

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CLP1-08

Study of the anti-*Listeria monocytogenes* effect of maqui (*Aristotelia chilensis*) extract at 8°C and 37°C

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Background. *Listeria monocytogenes* (*Lm*) is a Gram-positive, ubiquitous and psychrotrophic bacteria, capable of forming biofilms that give resistance to disinfectants and sanitizers, for this reason new control strategies are required. The use of bioactive compounds with antimicrobial capacity are presented as an alternative. These compounds are found in plants that have a high polyphenolic content, such as maqui (*Aristotelia chilensis*). This fruit has demonstrated antimicrobial properties against microorganisms such as *S. aureus* and *E.coli*. The aim of the present study was to evaluate the antimicrobial effect of maqui extract (ME) on *Lm* strains. **Methods.** The methanol extract at 80% was prepared with lyophilized maqui which was previously characterized by its oxygen radical absorbance capacity (ORAC) and total polyphenolic compounds. The study includes 43 *Lm* strains isolated from food and environmental samples. The anti-*Lm* effect of ME was assessed at 37°C through dilution agar assay (DAA) with different concentrations of the extract (0.05%, 0.1%, 0.15%, 0.2% and 0.25% v/v). The minimum inhibitory concentrations of maqui (MIC-maqui) were defined as the lowest ME concentration that inhibit the bacterial growth. Then, 2 strains (LIST 2-2 and BS 3-2) were chosen to evaluate the anti-*Lm* activity through DAA at 8°C. **Results.** The ME inhibited 100% of the strains, but we found different percentage of inhibition. The numbers of strains inhibited at 37ºC with different concentrations were 2 at 0.15% v/v, 15 strains at 0.2% v/v and 26 strains at 0.25% v/v between these is the reference strain EGD-e. Was not identified a relationship between MIC-maqui and source of strains. Results at 8ºC showed that ME has inhibitory effect over the strains evaluated, finding an equivalent effect that to observed at 37ºC. **Conclusion.** Methanol allows the extraction of anthocyanins that could be involved in the antibacterial effect. The maqui extract has anti-*Lm* activity at 37°C and the inhibition of the extract is kept at low temperature. Therefore, maqui bioactive compounds possess antimicrobial properties against *Lm*, allowing it to be considered as a food control alternative. In a future we will evaluate the effect of maqui in *Lm* biofilm production at 37°C and 8°C.

FONDECYT 117157 Enlace 018/16

CLP1-09

Pirin-mediated epithelial-mesenchymal transition in cervical cancer cells exposed to curcumin

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Background. High-risk human papillomavirus type 16 and 18 are the main causative agents of cervical cancer (CC) by the expression of E6 and E7 oncoproteins. In addition, it has been reported that these viral proteins are involved in the activation of epithelial-mesenchymal transition (EMT). Curcumin is a natural polyphenol compound that reduces E6 and E7 expression and EMT, although the mechanism by which this occurs is unclear. We propose that the decrease of EMT in cervical cancer cells exposed to curcumin occurs by a mechanism mediated by pirin. This protein works as an oxidative stress sensor and is overexpressed in an E6/E7-dependent manner. Thus, the objective of this study is to evaluate the role of curcuminin pirin-dependent EMT in CC cells. **Methods.** The CC tumor cell line SiHa (HPV-16 positive) was exposed to curcumin under different conditions. To evaluate the response of SiHa cells to curcumin exposure, the MTS assay was performed with a time-kinetics at 24, 48, 72 and 96 hours. Subsequently, we also evaluated the expression of EMT-associated biomarkers and pirin at RNA and protein levels using RT-qPCR and western blot, respectively. **Results.** It was found a significant decrease of cervical cancer cells viability at 72 and 96 hours. E-cadherin levels were significant increased whereas that Snail, Slug and Zeb-1 were significant decreased at RNA and protein levels. Finally, pirin levels were significant decreased after curcumin exposure. **Conclusions.** These results suggest that curcumin regulates EMT with downregulation of pirin. The importance of pirin in curcumin-dependent EMT regulation needs to be confirmed.

CLP1-10

Synthesis of metal(loid) nanostructures with biotechnological potential using metal(loid)-reducing flavoproteins

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Flavoproteins play a redox function in the cell using a flavin group (FAD or FMN) electron donor. Adjacent to the flavin, there is a redox center that is required for the activity and consists basically of two vicinal cysteine residues. These proteins can transform soluble, toxic metal(loid)s into less soluble, less toxic derivatives. Our laboratory has identified *Escherichia coli* flavoproteins such as tyrosine reductase, alkyl hydroperoxide reductase, dihydrolipoamide dehydrogenase, among others, exhibiting the ability to reduce efficiently tellurite in a NAD(P)H-dependent manner, thus generating Te nanostructures (NS). NS are structures with one or more dimensions in a scale less than 100 nm. At this scale, matter behaves differently and acquires new properties. Because of this, the interest in using NS for different purposes has increased and currently there is a great interest to find environmentally friendly methods for NS synthesis, using both microorganisms and/or enzymes. In this work, we initiated a search for flavoproteins showing the ability to reduce tellurite, selenite, silver, copper and gold.The reduction of these elements resulted in NS generation, indicating that them could be useful as tool for recovering these metal(loid)s from solution. Different *E. coli* ASKA and BL21 strains were used to produce and purify six flavoproteins of interest. Reduction reactions were monitored spectrophotometrically at different pH values, using either NADH or NADPH and under anaerobic and anaerobic conditions. NS synthesis was carried out by incubating each flavoprotein under their optimal reduction conditions. Resulting NS were characterized by DLS, EDS, TEM and ICP-OES. While GorA and reduced tellurium, selenite and silver, AhpF reduced tellurite and selenite. The reduction products exhibited nanostructure properties.

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CLP1-11

Extraction of hydroxycinnamic acids from spent coffee ground by substrate solid fermentation

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Worldwide, in 2016, the industry produced $9.03x10^6$ tons of coffee, generating $6x10^6$ tons/year of a solid waste called "spent coffee ground" (SCG) The SCG is constituted by the insoluble husk of the grain and had a high percentage of water (~60%). The SCG has at least 2,3% chlorogenic acid (CGA), phenolic compound constituted by a molecule of acid quinic (QA) and one of acid caffeic (CA). The CGA is present in vegetables and the fruits of coffee being its main source. The three compounds are characterized for its antioxidant capacity. The aim of this work was to determine the concentration profiles over time of CGA, QA and CA (hydroxycinnamic acids) during its extraction from spent coffee ground by substrate solid fermentation (SSF) using a microbial consortium of the genus Aspergillus sp. The experiments were run in column reactors for 30 days at 35°C with aeration per bottom of 4 L/h. The phenolic compounds were extracted using a hydroalcoholic solution and quantified by HPLC. The unfermented SCG showed 1.79 mg CGA/g SCG in dry basis (db); 0.19 mg CA/g SCG (db) and 0.22 mg QA/g SCG (db). At day 12 of SSF, maximum CGA concentration was obtained, with an extract containing 7.2 mg CGA /g SCG (db); 1.3 mg CA/g SCG(db) and 1.19 mg QA/g SCG (db), a condition where the biomass was found to be in a sustained growth phase. At day 23 of the fermentation the maximum concentration of QA was presented, obtaining an extract with 1.6 mg CGA/g SCG (db); 4.7 mg CA/g SCG(db) and 5.1 mg.QA/g SCG(db), microorganisms were in stationary phase. Concentrations of 0.7 mg CGA/g SCG (db); 5.0 mg CA/g SCG(db) and 4.8 mg QA/g SCG(db) were detected at day 30. In conclusion, solid substrate fermentation of SCG showed greater extraction yields of phenolic compounds in comparison to conventional hydroalcoholic extraction and different concentration profiles were obtained for CGA, CA and QA along the time, being an alternative to produce phenolic compounds extracts with different compositions.

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CLP1-12

Metabolic engineering of *Pseudomonas putida* for tailor-made PHA synthesis with different properties and industrial applications

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Background. Polyhydroxyalkanoates (PHAs) are biopolymers displaying similar properties to oil-based thermoplastics. Many microorganisms accumulate PHAs as carbon and energy storage compounds in their cytoplasm under nutrient-limiting conditions and carbon excess. One of the leading PHAs produced at industrial scale is the co-polymer poly(3-hydroxybutyrate-valerate) (PHBV). This exhibits excellent thermoplastic qualities and a large spectrum of applications. The main limitation in the microbial production of PHBV is the use multiple substrates such as glucose, valerate, and/or propionate. However, the haloarcheon *Haloferax mediterranei* have been recently described to be able of synthesizing PHBV from glucose as the only carbon source. This unique feature is associated to the specificity of the enzyme PHA synthase. Nevertheless, the use of this microorganism for PHA production is limited by the low yield of PHA on glucose and high salt level required to grow *Haloferax*, where the latest speeds up the corrosion of metal components of the bioreactors. On the other hand, *Pseudomonas putida* KT2440 have been described as an efficient PHA producer using different carbon source such as fatty acids. Nevertheless, the PHBV production in this strain is restricted by the specificity of its own PHA synthase. Through a genetic engineering approach, we have modified *Pseudomonas putida* to allow the synthesis of PHBV co-polymers. **Methods.** A PHA synthase (*phaC1-phaC2*) and PHA depolymerase (*phaZ*) knock-out mutant strain have been constructed utilizing the I-SceI system, based on homologous recombination. The resulting mutant was used as host to overexpress the PHA synthase (*phaEC*) from *Haloferax mediterranei.* The heterologous expression was carried out using restriction enzymes and the expression plasmids pSEVA228. The PHA producing capacity of the different modified strains has been performed utilizing decanoate and nonanoate as carbon sources. The composition of PHA produced was characterized by gas-chromatography coupled to mass-spectrometer. **Results.** In this work we have generated a strain of *P. putida* KT2440 able to synthesize the co-polymer PHBV trough the *phaEC* synthase expression and fatty acids degradation. **Conclusion.** The metabolic engineering of *P. putida* leads to the modulation of tailor-made PHAs production with different properties and industrial applications.

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CLP1-13

Biological soil-crusts (BSC) forming cyanobacteria favour plant germination and improve the quality of soil contaminated with mining residues

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Biological Soil Crusts (BSC) are biotic communities formed after cyanobacterial colonization. A mix of two BSC-forming cyanobacteria isolated from environmental samples, *Trichocoleus* sp. and *Leptolyngbia* sp., was inoculated on substrates composed by a mix of soil and copper mine-tailing sands. The establishment of the cyanobacteria on the soil mixes was observed by optical and confocal microscopy. The results showed that the cumulative germination of the plants was significantly higher in the presence of the cyanobacteria than in the controls. A similar result was observed for the growth of *P. australis* in the presence of cyanobacteria, showing stimulation of the plants growth respect to the controls. The organic matter content (OM) increased after the inoculation of the soil mix with the microorganisms, and the electrical conductivity (EC) was alleviated due to the cyanobacterial inoculation. The inoculation of mining polluted soil with BSC-forming cyanobacteria diminishes the phytotoxicity of the substrates and may favours the establishment of plant species.

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CLP1-14

Another one bites the dust: Microbial consortia generation for arsenic treatment in flue dust from mining process

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Nowadays, the recovery of metals from low-grade ores and the treatment of mining wastes are one of the permanent challenges in the mining industry. One way of extracting metals from mineral resources that has been slightly developed by conventional mining approaches is the use of methods based on microbial capabilities. The Northern of Chile has a variety of poly-extreme ecosystems, characterized with high solar radiation, low relative humidity, extreme temperature variations and the presence of high concentrations of metals (eg. Cu, Fe, Au, Ni). Nevertheless, the microbial life can develop under these challenging environmental conditions, which makes them an ideal place for the search of extremophilic microorganisms with specific physiological and metabolic adaptations. The purpose of this study is to identify and characterize a microbial consortium based on samples from a high altitude Andean wetland located at the Tatio Geothermal Field, capable of tolerating, oxidizing and/or reducing different arsenical species, which can be used for the abatement of arsenic applied to mineral residues rich in metals of interest that allow their subsequent recovery (flue dust). Based on analyzes of high quality 16S rRNA gene sequences obtained by High-Throughput Sequencing Technologies from sediment and microbial mat samples, we develop different culture strategies to enrich the most promising microorganisms that could actively participate in the oxidation of certain minerals and metalloids. Moreover, these analyzes allowed to categorize the main microbial groups in such extreme environments, being mainly groups belonging to the Phylum Firmicutes, Nitrospira and Proteobacteria. In order to determine the level of interaction of the microorganisms with the metals of interest, different tests with variation in pH, metal concentrations (arsenic and iron), as well as concentration of mining wastes (flue dust and PLS) were performed. Finally, the oxidation rates of iron and arsenic at the beginning and at the end of the enrichment culture were determined by chemical analysis. This work grants a technological proposal for the valorization of unique environments of the North of Chile, presenting an alternative methodology for the treatment of mining contaminants.

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CLP1-15

Differential expression analysis of metabolic genes of *Sulfobacillus thermosulfidooxidans* under different growth conditions

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Background. Microorganisms that inhabit extreme environment like bioleaching heap processes have developed many strategies to resist difficult growth conditions such as high temperatures, acidity, high concentrations of metals, toxic solvents, among others. The study of gene expression associated to different metabolisms allows us to identify and understand the mechanism that those microorganism uses to grow in such environments. In this work we present a transcriptomic study performed under different growth conditions in the gram positive bacteria *Sulfobacillus thermosulfidooxidans CBAR13* that inhabits the heap bioleaching from Escondida Mine. This bacteria is an acidophilic, moderated thermophilic that uses ferrous ion and reduced sulfur compounds as energy source. The aim of this work was to identify genes differentially expressed when the microorganism grow through the use of two different energy sources. **Methods.** *Sulfobacillus thermosulfidooxidans CBAR13* culture was grown using iron or tetrathionate as energy source. We extracted RNA when the bacteria was in its exponential phase of growth in each culture and performed a massive RNA sequencing. The data from the sequencing was processed with the software CLC Genomics Workbench and the analysis of count data for the detection of differentially expressed genes was made using the *DESeq2* package from the informatics tool for analysis of data "R". **Results.** We found 575 genes overexpressed in the culture grown on iron, 12 of them are associated to iron oxidation. 410 genes encode hypothetical proteins. Moreover, in the tetrathionate growth condition, we found 703 genes overexpressed, 13 of them associated to sulfur oxidation. 432 genes encode hypothetical proteins. **Conclusion.** The identification of genes that are differentially expressed depending on the growth conditions of the bacteria allows the increase of the knowledge on the metabolisms to which these genes are associated like iron and sulfur oxidation, two fundamental metabolism in the bioleaching processes. Some of them were previously described as related to the dissimilatory iron oxidation metabolism for microorganisms like *A. ferrooxidans*. However, some other overexpressed genes have an unknown relation with iron oxidation. This open a window of opportunity for the discovery of new components of the energetic metabolisms in *S. thermosulfidooxidans*.

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CLP1-16

Evaluation of the response of genes associated with oxidative stress and its relation to the mechanism of resistance to p-coumaric acid in *Brettanomyces bruxellensis*

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Brettanomyces bruxellensis has been described as the main yeast contaminant of the wine, due to its ability to metabolize naturally occurring *p*-coumaric acid in the grape to less toxic compounds. Recently, transcriptomic studies have reported that the mechanism of resistance to *p*-coumaric acid by *B. bruxellensis* is strain-dependent and occurs mainly during the *lag* phase of the growth curve. The results allowed to propose a model of early response to *p*-coumaric acid stress. Its entry into the cell causes a generalized stress condition, which induces the expression of proton pumps and mechanisms involved in the outflow of toxic compounds.Also, genes related to oxidative stress response were found to be strongly overexpressed. In this study, the relative expression of genes related to oxidative stress by RT-qPCR in two strains of *B. bruxellensis* (LAMAP1359 and LAMAP2480) that showed differences in *lag* phase duration when exposed to *p*-coumaric acid were compared. Later these genes validated by *Pichia pastoris* GS115 transformation. The results indicate that there is a greater expression of the *SOD1, GCN4, HSP12* and *SFL1* genes in the strain of *B. bruxellensis* LAMAP2480 when compared with the strain LAMAP1359. On the other hand, *ESBP6* gene has a greater expression in the strain LAMAP1359 the *CCP1* gene does not present significant differences between both strains. In evaluating the growth kinetics of the *P. pastoris* GS115 transforming strains, it was observed that the presence of a copy of the analyzed gene improves the growth rate compared to the untransformed strain grown in the same condition.

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CLP1-17

Marine actinobacteria from Chilean coasts: a prospective source for the discovery of keratinases for application in poultry processing industries

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Feathers are produced in large amounts as a waste by-product of poultry processing plants. A current value-added use for feathers is the conversion to feather meal for animal feed, using physical and chemical treatments. These methods can destroy certain amino acids and decrease protein quality and digestibility. To overcome this situation, microbial treatments are being considered with varying degrees of success. Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin. Keratinases from bacilli, fungi, and actinomycetes have been studied for effective degradation of keratinous waste. The *phylum* Actinobacteria is recognized for its potential in the production of bioactive compounds and commercial enzymes. Actinobacteria are one of the most diverse groups of bacteria in nature, are present in various ecological habitats including marine environments. Marine actinobacteria are able to produce enzymes with novel physiological characteristics, such as high salt tolerance, thermostability, barophilicity, and cold-activity, constituting an attractive source of enzymes of industrial and/or environmental interest. Previous studies have shown that the Chilean coast is a favourable ecosystem for the search of marine actinobacteria with great potential for the production of bioactive compounds and enzymes. Seventy-three bacterial strains belonging to our Chilean marine actinobacterial culture collection, isolated from marine sediments, sponges and a sea urchin obtained from the coast of Chile ranging from the III to the XI Region, including Easter Island, were analysed for keratinase activity. Isolates were screened for their ability to hydrolyse keratin in turkey feather agar plates, measuring the colony diameter. The strains which exhibited a large hydrolysis zone, were grown in liquid culture medium containing feather as carbon and nitrogen source. In the course of the culture growth, the enzymatic activity, degree of hydrolysis and percentage of degradation was measured. The strains that presented higher enzymatic activity belong to the genera *Streptomyces* and *Actinomadura.* In the present work, we report marine keratin-degrading actinomycetes and its ability to degrade turkey feathers. Keratinolytic actinobacteria from marine habitats could open new opportunities for the discovery of novel keratinases for application in poultry processing industries.

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CLP1-18

Characterization of biofilms and bioleaching on pyrite grains by *Leptospirillum ferriphilum*

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Bioleaching is the process of oxidation of metal sulfides to soluble ions by certain bacterial and archaeal species. Bioleaching microorganisms can be found in acid mine drainages and in biomining operations. One of the key steps of this process is the biofilm formation, which is mediated by the production of microbial extracellular polymeric substances (EPS) that structures the biofilms formed by leaching microorganisms on mineral surfaces. *Leptospirillum ferriphilum*, is a moderate thermophile able to oxidize iron and, consequently, some metal sulfides such as pyrite as unique energy source. It generally shows a dominant role on microbial communities and biofilm formation. Previous work has demonstrated that *L. ferriphilum* is able to attach to different surfaces, including pyrite coupons. The objective of this work is to characterize biofilm formation with a more realistic approach by utilizing pyrite grains, instead of flat coupons. In this work, we have characterized *L. ferriphilum* attachment to pyrite, over time directly, by visualizing biofilm formation by epifluorescence microscopy using nucleic acid fluorescent dyes, and as well as indirectly by measuring the planktonic cell concentration by cell counting during leaching assays. Besides we have been able to identify suitable binding lectins to recognize one single species biofilm. Also, total iron has been measured during pyrite leaching assays using phenantroline-iron determination method. Our results show that ~80% of a *L. ferriphilum* attached to pyrite grains within the first 4 h. We also followed biofilm formation during time and observed pyrite pitting due to microbial oxidation. These studies set the base for further microscopy and leaching studies of interactions within *L. ferriphilum* and other leaching bacterial during biofilm formation in metal sulfides, which are in progress.

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CLP1-19

Genomic insights into the biotechnological applications of the arsenic-tolerant Actinobacterium *Rhodococcus erythropolis* S43.

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Rhodococcus erythropolis S43 is an actinobacterium isolated from an arsenic-contaminated soil sample, collected from an old smelter site, in Germany. This strain has unique features as compared to the other members of the species, namely resistance to elevated concentrations of arsenic. Here, we present the microbiological features and genomic properties of this biotechnologically relevant strain. The 6,812,940 bp draft genome is arranged into 264 scaffolds of 848 contigs. It possesses 62.5% of CG content and comprises 6,040 coding sequences and 49 tRNA genes. Bioinformatic genome analysis showed the presence of arsenic-resistance genes. A complete *ars* operon was found containing the *arsACDOR* cluster coding for ArsAB (efflux pump and ATPase), ArsC (arsenate reductase), ArsD (chaperone) and ArsR (*ars* operon regulator). Our results show that the *arsC* mRNA level significantly increased in response to arsenite and arsenate exposure, suggesting its involvement in the arsenic resistance phenotype of strain S43. In addition, this strain showed to have a plethora of genes coding for proteins involved in oxidative-stress response, including catalase, super-oxide dismutase, glutathione peroxidase-related genes, thioredoxin and thioredoxin reductase, suggesting it is highly tolerant to oxidative conditions. Finally, genes for radiation resistance, biodesulfurization, and oil and phenol degrading pathways were also detected. Altogether this data make *R. erythropolis* S43 a good candidate microorganism for bioremediation of highly contaminated environments and other industrial applications.

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CLP1-20

Unveiling the viral diversity of Antarctic coastal waters and their representation in global viral estimates

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Viruses are the most abundant biological entities on the ocean (1), and they influence microbial community composition and abundance, metabolic activity, and evolutionary pathways (2,3) and therefore have a major role in biogeochemical cycles. Despite their importance, viral diversity remains poorly understood with less than 1% of the total diversity currently isolated and constrained to cultivable hosts. The recent massive sequencing of double-stranded DNA viromes in expeditions such as Tara Oceans and Malaspina have successfully overcome this bias, resulting in a global map of the ocean virome -GOV- (4). The Southern Ocean however, is consistently underrepresented on global analyses like those ones. This marine region is one of the most productive areas of the ocean, it accounts approximately 20% of total carbon drawdown were almost the totality of this is carried out on the summer season at coastal waters. Here we present a study of the summer functional and taxonomical viral diversity from Chile Bay, West Antarctic Peninsula, by high throughput sequencing. We retrieve approximately 500 contigs (>5kb) or complete circular genomes from our metavirome. Total proteins were predicted and clustered using the Markov clustering algorithm (5), to assess functional diversity. Between the most abundant proteins, several viral structural motifs and accessory genes were identified. Then, we used the protein content of contigs and classified them using the vConTACT tool. We obtain 48 viral populations, where four populations concentrate over 50% of total classifiable virus. Phylogenetical analyses of predicted capsid protein sequences assign taxonomically our identified viral populations. Comparisons between our more abundant protein clusters and viromes from other Antarctic coastal waters against those previously reported on the epipelagic and mesopelagic waters on a global scale demonstrated that most abundant protein clusters across the ocean did not necessarily correspond, neither structurally or their metabolic accessory genes, with the ones observed in Antarctic coastal waters. These results suggest that the functional local viral diversity of Southern Ocean coastal waters still remains under represented on global considerations and therefore their impact on global biogeochemical balances is being underestimated.

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CLP1-21

Diversity and structure analysis of Actinobacteria and characterization of *Streptomyces* isolated from Ñadi soils

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The soil is the most important component of the biosphere, since it sustains life of a vast amount of species, among which bacteria stand out, taking part in several biogeochemical processes. Soil conformation varies around the world, and in southern Chile, the most prevalent soils belong to the Andisol type and are commonly called Trumaos and Ñadis. These soils are volcanic in origin and usually waterlogged. Among the bacteria living in the soil, those of the order *Actinomycetales* (actinobacteria) generate secondary metabolites with biotechnological potential, and particularly, bacteria of the genera *Streptomyces* produce around 70% of these metabolites. In Chile, there are no studies about actinobacteria on Ñadi soils. The goal of the present study was to prospect the diversity of actinobacteria and *Streptomyces* species in five series of Ñadi soils using PCR-DGGE and bacterial culture. For culture-independent techniques (PCR-DGGE), specific primers for actinobacterial 16S rRNA gene were used. Moreover, pH was determined in each soil series, since it's an influential factor in actinobacterial development. In order to isolate *Streptomyces*, three different culture media were used, isolates were Gram stained and several carbon source utilization tests were carried out to compare the isolates with strains described in Bergey's Manual, 8th Edition. The Shannon-Weaver diversity index (H') for actinobacteria was low on each site (≈1.59). Notably, it was found that *Streptomyces flavogrisens*, a bacterium that produces antimicrobial compounds Actinomycin D and Holomycin, was present in every soil. In conclusion, despite their low diversity, actinobacteria were present in every sampled soil series. Future studies are required and should be focused on exploring the biosynthesis of new secondary metabolites from these bacteria, with potential biotechnological use.

DID-UACh 1310-32- 33 Exploración de potenciales aplicaciones biotecnológicas de microorganismos aislados desde suelos de la región sur-austral de Chile

CLP1-22

Genotypic and phenotypic characterization of native PGPR isolated from agricultural soils contaminated with metals

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Background. Plant growth-promoting rhizobacteria (PGPR) are bacteria capable of promoting plant growth by colonizing the plant roots. These bacteria were mainly used for assisting plants to uptake nutrients from the environment or preventing plant diseases. Recently, the application of PGPR has been extended to bioremediation of both organic and metal contaminated soils in association with plants. In a previous study, bacterial strains with PGPR properties and copper resistance were obtained. The aim of this study was to identify and characterize these native PGPRs isolated from copper-contaminated agricultural soils. **Methods.** Nitrogen fixation, siderophores and indole acetic acid production were evaluated in strains S6C1 and S32C2. In addition, swimming and swarming motility were studied. 16S rRNA sequencing was used for bacterial identification and strains were examined for the presence of plasmids. **Results.** Molecular identification by 16S rRNA sequencing suggested that S32C2 strain belongs to the genus *Leclercia*, while S6C1 strain to the genus *Rhizobium*. *Leclercia* sp. S32C2 contains two plasmids, in one of which copper-resistance determinants were localized (*copA* gene). Only one plasmid was detected in *Rhizobium* sp. S6C1. Both isolates were capable of fixing nitrogen and produced siderophores and indole acetic acid. *Leclercia* sp. S32C2 showed swimming and swarming motility. **Conclusion.** *Leclercia* sp. S32C2 and *Rhizobium* sp. S6C1 showed plant growth promoting capabilities and resistance to copper. These results suggest the possibility to use these rhizobacteria as a promising microbial inoculant for increased plant growth in metal-contaminated soils. Interesting features were observed in *Leclercia* sp. S32C2, where copper resistance was found to be probably encoded by plasmids. Further studies are needed to confirm the nature of these genetic determinants.

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CLP1-23

Microbial diversity at the limits of life in nitrate/iodine deposits in Northern Chile

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The Atacama Desert has been described as a Salt Domain and provides the setting for the unique economic nitrate/iodine ore deposits. These kind of extreme environments are unique habitats that allow us to find microorganisms with scientific and technological potential interest. The deliquescence of hygroscopic minerals, such as chloride salts, could provide a local and transient source of liquid water that would be available for microorganisms on the surface. The present work investigated how the saline facies interact with microbial communities present at the Atacama Desert, in the nitrate/iodine ore deposits. The main goal is to identify the microbial diversity in selected saline facies in Northern Chile. In order to identify the taxonomy and phylogeny of the microbial communities occurring in these facies, DNA samples of soil surface were obtained from Oficina Pissis (OP) and Oficina Savonna (OS) in the Domeyko Group. The microbial community analyses were carried out by sequencing the 16S rRNA clone library by Illumina Myseq. Analysis of high-throughput community sequencing data was carried out through the QIIME software. Primer-6 was used to calculate diversity indexes, and PICRUSt was used to perform functional predictions. Diversity indexes reflected the heterogeneity of the communities, samples with the higher diversity, based on the number of species and their relative abundances, were found in OP. The most samples from this site showed the presence of hygroscopic nitratine and halite minerals, which may support the presence of microorganisms, possibly associated to nitrate reduction.The microbial communities in these facies were dominated by Proteobacteria and Firmicutes. For OS samples, the predominant microorganisms were Betaproteobacteria, while in OP samples were Bacilli, followed by Clostridia. The Archaea diversity was also dominated by Euryarchaeota, *Halorobrum tebenquichensi* and the Crenarchaeota, *Nitrosopumilis maritimus*. The study of the microbial diversity associated to these different saline facies, such as nitrate and perchlorate, is relevant for the potential scientific impact and the habitat sustainability in arid and hyper arid environments. These environments can be considered analog environments to early life in Mars, due to evidence of microbial participation in the biogeochemical nitrogen cycles in these unique nitrate reservoirs.

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CLP1-24

Characterization of Thalassiosirales members from Antarctic phytoplanktonic communities

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Antarctic coastal regions are highly productive ecosystems, characterized by unique environmental conditions, as temperatures below the freezing point, complete darkness in winter and continuous day light and high UV radiation in summer. In these systems, photosynthetic microorganism, especially diatoms, are the base of the food web, with recurrent spring and summer blooms that contribute in high proportion to carbon fixation and intake. Diatoms inhabiting this environment have adapted their physiology to maintain growth, reproduction, and metabolic activity in these extreme conditions, and because of that, in this work we aim to analyze the (meta)genomic features allowing Antarctic diatom communities to thrive in this environment. Metagenomic analysis of samples taken during a short summer bloom in January 2013, at Bahia Fildes, Antarctica, and belonging to three size fractions (0,2-3 µm, 3-12 µm, and 12-20 µm) shows that: i) the free-living size-fraction sample (0,2-3 µm) was dominated by Bacteria, with ~98% of HQ sequences, mainly belonging to Gamma- and Alphaproteobacteria. The most abundant groups were SAR11 clade and Oceanospirillales, in agreement with the scarce previous studies. The two other fractions were dominated by sequences classified as Eukarya corresponding to ~50% of ribosomal sequences in the 3-12 µm size fraction and ~88% of the 12-20 µm size fraction, respectively. The major eukaryotic group in both size fractions was Stramenopiles, being diatoms belonging to Thalassiosirales the most abundant order. ii) Functional predictions shows a global dominance of heat shock proteins, DEAD- box, RNA helicases and fatty acid desaturases: These functions could be an evidence that metabolism in this Antarctic microbial community is low-temperature-adapted, with biosynthesis of compounds and energy metabolism as the most dominant metabolic pathways. This work shows the first metagenomic analysis of pico- and nano-plankton communities from Antarctic waters, integrating bacterial and microbial eukaryotes.

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CLP1-25

Mapping *Acidithiobacillus* populations with high resolution onto a natural environmental gradient

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The Copahue-Caviahue-ChanchoCo geothermal system is a natural extreme environment located in the Andes mountain range of northern Patagonia, right at the border between Chile and Argentina. Two hydrothermal springs near the Copahue volcano crater are the source of the acidic Río Agrio, a watershed of unique characteristics in South America. Along its journey from the source to Lake Caviahue, and downstream of the lake for an additional 40 Km, the river features an ample gradient of pH (pH 0.5–8.5), temperature (10-80ºC) and conductivity (0.5-500 mS/cm). Several fumaroles, pools, ponds and hot springs away from the river path complete the system. The wide range of physical and chemical conditions measured in this environment support a great biodiversity of algae, archaea and bacteria. Several taxons of extreme acidophilic bacteria have been found to occur along this environmental gradient, including sulfur-oxidizers of the Acidithiobacillus species complex. In this study, the microdiversity of Acidithiobacillus populations inhabiting the Copahue-Caviahue-ChanchoCo geothermal system is explored using culture-dependent and independent strategies. Phylogenetic and oligotyping analysis of the 16S rRNA gene sequence is used to infer the taxonomy of the representative isolates and sequence clones and to resolve meaningful differences between closely related populations along the environmental gradient. The correlation between the emerging population structure and the physico(geo)chemical gradient is analysed through multivariate statistical analysis in order to gain insights into the ecological and evolutionary processes that structure and maintain microbial diversity in this ecosystem.

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CLP1-26

First report of *Botrytis cinerea* causing gray mold disease on the endemic plant *Echinopsis coquimbana* in the Coquimbo Region, Chile

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Botrytis cinerea is a phytopathogenic fungus which infects different plant species and can cause significant crop losses in local and worldwide agricultural industries. However, its presence in the endemic flora in the IV Region of Coquimbo, as well as its impact on local flora has not been studied yet. *Echinopsis coquimbana* is a member of the Cactaceae family and is widespread and abundant along Coquimbo's coast (Rundel *et al*. 1998). Our studies were conducted in June 2014 at a single location near 'Caleta Hornos' at 36 km northern La Serena, Chile. (29º 37' 54.48'' S 71º 17' 03.04'' W). Findings revealed that 10 % of *E. coquimbana* plants displayed disease symptoms on their spines base resembling gray mold disease. Diseased spine tissues were excised, and the surface was disinfected by immersion in 1% NaOCl for 60 sec, placed on malt extract agar (MEA, 2% malt extract and 2% agar), and incubated at 22°C. Fungal colonies obtained were initially white, then became gray to brown after 6 days. Analysis of light micrographs displayed elliptical conidia on MEA were 7.9 to 12 µm long and 6.4 to 9.5 µm wide (n = 300). The ITS region of rDNA was amplified using ITS1/ITS4 primers and then sequenced. ITS analysis of this isolate revealed that it corresponds to genus *Botrytis*. For further confirmation, nuclear protein-coding genes (G3PDH, HSP60, and RPB2) were sequenced (GenBank ASS KY798122-124). Outcoming sequences showed 100% identity against *B. cinerea*. Isolate Bc.eq01 was used to conduct pathogenicity tests in tomato fruits and leaves, as well as in *E. coquimbana*. Both, tomato leaves and fruits developed typical gray mold symptoms after 4 days of inoculation, similar symptoms were observed in *E. coquimbana,* and no symptoms were observed on control plants. Results provided here contribute to our fundamental understanding of the presence of *B. cinerea* in an endemic plant from the IV region in Chile, as well as the potentially adverse effects on local agriculture and the development of endemic plants (as potential *Botrytis* natural reservoir). Further studies are required to determine the distribution and ecology impact of this disease on *E. coquimbana.*

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CLP1-27

Cultivable fungi associated with marine sponges from Collins Bay and Rada Covadonga, Antarctica

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Despite fungi play an important role in different ecosystems, the diversity and the biogeographical patterns of fungi remain poorly understood. In particular, the fungal biodiversity of the Antarctic marine ecosystems is practically unexplored. In a previous work, we described the cultivable biodiversity of filamentous fungi associated with Antarctic marine sponges from Fildes Bay, King George Island, Antarctica. In the present work, we extend our study to other two geographical locations: Collins Bay (King George Island) and Rada Covadonga (Antarctic Peninsula). Fifty three fungal isolates were obtained from nine sponges collected in Collins Bay, whereas twenty seven fungal isolates were obtained from six sponges collected in Rada Covadonga. The phylogenetic analysis of these isolates using ITS sequences revealed that they are representative of three genera: *Aspergillus* (family Trichocomaceae), *Penicillium* (family Trichocomaceae) and *Pseudogymnoascus* (family Pseudeurotiaceae). Interestingly, the distribution of these genera is different and depends on the sampling location. Thus, *Penicillium* is the predominant genus found in sponges from Rada Covadonga, whereas *Pseudogymnoascus* spp. are prevalent in sponges from Collins Bay. The phylogenetic analysis of the *Pseudogymnoascus* isolates shows an unexpected diversity within this genus. Finally, our analyses also suggest that several isolates of *Pseudogymnoascus* would be non-described species. This work will help in our understanding of the fungal distribution patterns in marine Antarctic environments.

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CLP1-28

Changes in the distribution of viral communities in aquatic biomes, unveiled through massive metagenomics

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In the last decades, increasing evidence has shown that bacteriophages across different environments are a vital part in ecological terms, as a driver of the dynamics of microbial communities, and in molecular terms, as a reservoir of genetic diversity. In aquatic environments, estimations indicated that nearly 90% of biomass is composed of viruses and daily, they eliminate approximately 20% of bacterial biomass. However, the dynamics and behavior of viral populations and its most abundant representatives still remain poorly understood. Currently, major questions in viral ecology are: How do those viral populations change among different aquatic environments (biomes)? Which ecological parameters are affected by those changes: average abundance, diversity, prevalence?, and not least, what phages are ubiquitous and which genetic characteristics gives them an advantage in order to adapt to diverse environments? The research proposed here addressed those questions by leveraging the genomic information collected by 420 previously published metagenomes extracted from aquatic environments around the world. Initially, we searched phage-related genes subjecting metagenomes to alignment using two phages databases, Phantome and Uniprot. Afterwards, we obtained meaningful metrics based in the proportion of reads homologous to phage-related genes for each of the three different aquatic biomes: marine (140 metagenomes), estuarine (140 metagenomes) and freshwater (140 metagenomes) biomes. Our analysis revealed that those viral species with higher abundance in specific environments, were also present in a greater number of biomes (prevalence). A large proportion of the viruses classified as more abundant falled in two main families, Myoviridae and Siphoviridae; some of those are ubiquitous in all the biomes (such as Prochlorococcus phage SSM-2, for example). In contrast with phages in less abundance, the genomes of those prevalent phages contain an enrichment of highly abundant genes, identified as auxiliary genes, suggesting that such genes may act like evolutionary tools for their success in the environment.

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CLP1-29

Hra family adhesins in enterotoxigenic *Escherichia coli*

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Background*.* Enterotoxigenic *Escherichia coli* (ETEC) uses a diverse set of adhesins to colonize the human intestine. Currently, there is no vaccine against ETEC and gaining insights into the adhesins repertoire is important for the development of preventive therapies. In this scenario, fimbrial structures have been the most studied until now. In contrast, non-fimbrial adhesins have been less explored. The Tia protein (encoded by the ETEC invasion locus A), related to the heat resistant haemagglutinin (Hra-1), has been one of the few cases. In this work, we looked for the genes that encode the other four representatives of the Hra family, Hra-1, Hra-2, Hek, and Hes in ETEC genomes available in databases. **Methods.** *E. coli* genomic sequences available in the NCBI refseq database were retrieved (6,036 genomes). Screening for ETEC marker genes, encoding heat-labile and heat-stable toxin genes, and Hra-family genes was performed with Large scale-blast score ratio using tblastn. Further alignments were performed with Clustal Omega and UGene. Functionality of the identified genes was determined by expressing them in the low adherent *E. coli* HB101 strain and assessing the capacity to agglutinate erythrocytes, to autoaggregate and to adhere and invade Caco-2 cells. **Results**. 385 genomes of human ETEC were identified. Among them, *hra-1* was detected in 36 genomes, *hra-2* in 15, and *hek* in seven. The *tia* gene, whose functionality had been probed in the prototype ETEC H10407 strain, was detected in 52 ETEC genomes. Representative ETEC strains harboring *hra*-family genes were selected from our local collection. Even when some point substitutions were identified in the deduced amino acid sequences, in comparison with the originally described proteins, all of the genes were able to confer adhesion capacity to Caco-2 cells when expressed in *E. coli* HB101. In addition, as was previously reported, expression of *hra-1* and *hek* conferred to this strain the capacity to form self-aggregates and to aggregate erythrocytes. **Conclusion**. Hra-family members Hra-1, Hra-2 and Hek are part of the ETEC virulence repertoire, although their presence was detected in low frequency.

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CLP1-30

Effect of 5-azacytidine on the expression of genes from the secondary metabolism in the Antarctic fungus *Pseudogymnoascus* sp.

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Filamentous fungi from the genus *Pseudogymnoascus* are prevalent in Antarctica, and several reports suggest that strains of this fungal genus are promising producers of secondary metabolites with novel chemical structures. Despite this, the secondary metabolism of *Pseudogymnoascus* sp. has been poorly studied. In a previous work, we obtained four DNA fragments from polyketide synthase (PKS) genes, and one DNA fragment from a non-ribosomal peptide synthetase (NRPS) gene. In this work, we show the study of the transcriptional pattern of these genes and the effect of the compound 5-azacytidine (5-AZA), an inhibitor of DNA methyltransferase function, on such expression patterns. The four PKS genes are expressed in the conditions assayed. However, expression of NRPS gene was not observed in any condition. After the addition of different concentrations of 5-AZA to the culture broths, we observed that three PKS genes changed their expression pattern, whereas one PKS gene was insensitive to 5-AZA. Finally, the NRPS gene was neither expressed in any concentration of 5-AZA used. Our results, particularly the change in expression of three PKS genes, will allow the future purification of the secondary metabolites associated with these genes from *Pseudogymnoascus* sp.

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CLP1-31

LuxS *quorum sensing* system in *Bifidobacterium dentium*

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*Bifidobacterium s*pp. are microorganisms anaerobic, Gram positive, acidogenics and acidurics, that inhabit the gastrointestinal tract and oral cavity. Species as *B. dentium* are considered etiological agents of dental caries, being associated principally with deep caries sites. With other microorganisms, *B. dentium* are able to form a biofilm on the dental surface, named dental plaque. In this biofilm, different bacterial species can interact through quorum sensing. In some Gram positive oral bacteria, the autoinducer-2 quorum sensing system has been well characterized. Autoinducer-2 is produced by LuxS enzyme and has been shown to have a role in grown, biofilm formation and acid resistance in those microorganisms. In *B. dentium*, few information of this system exists. The aim of this study was analyze the presence and functionality of LuxS quorum sensing system in *B. dentium* ATCC 27534, a caries dental isolated microorganism. For this purpose, the presence of *luxS* gene was determined by bioinformatics and PCR amplification. Levels of its mRNA were analyzed through RT-PCR, in different grown phases, and no differences among them were found. On the other hand, using the *V. harveyi* BB170 reporter system, the presence of autoinducer-2 was determined in supernatant of *B. dentium* cultures, indicating that this microorganism is able to produce it. Finally, effect of autoinducer-2 in adhesion and biofilm formation of *B. dentium* was analyzed. Adhesion assays were performed on 96-well polystyrene microplates in presence or absence of chemical autoinducer-2, using saliva and culture medium, by 16 hours. Bacterial adherence was measured using the MTT assay and a SynergyHT (Biotek®). Biofilm formation was measured at the same conditions as adherence assay, for 3 and 5 days, and documented with a scanning electron microscope (JSM-IT300, JEOL). In presence of autoinducer-2, a significant increase was shown, either in adhesion or biofilm formation, indicating that this quorum sensing system is important to control these physiological processes in *B. dentium*. Further research will allow us to elucidate how these microorganisms communicate with each other through this system, as well as their participation in the cascade of regulations that allow the permanence of *B. dentium* in the caries sites.

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CLP1-32

Transcriptional changes of genes regulated by *yqgF* gene product in *Salmonella* Typhimurium

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Termination of transcription in prokaryotes is a highly-regulated process, mainly for two reasons: to avoid interferences with transcription of neighbor genes and the expression of foreign genes that are harmful to bacterium. There are two mechanisms of transcription termination, one is the Rho-dependent. Rho is an essential factor in enterobacteria, acting on overall regulation of gene expression in conjunction with other cofactors. A new factor affecting Rho-dependent termination is YqgF. Its biological and biochemical function is unclear and has been reported to be essential in *S*. Typhi and *E. coli*. Recently YqgF has been involved in anti-termination of internal Rho-dependent sequences. In *E. coli* expressing a thermos-sensitive copy of *yqgF* it was found that YqgF participates in the processing of pre-16S rRNA, producing defective ribosomes, which the authors speculate bind shine-dalgarno (SD)-like sequences strongly, causing pauses in translation and consequent decoupling of transcription, especially of genes with high content (≥6) of SD-like sequences. In *Salmonella* Typhimurium the role of YqgF has not been described and preliminary studies indicate that is essential. We analyzed how expression of *yqgF* affects growth of *S.* Typhimurium. Considering that Rho protein plays a key role in regulation of SPI-1 and SPI-2 genes, implicated in invasion and proliferation of bacteria, and that many of those genes contains many SD-like sequences, we analyzed the expression of these genes with high and low expression of *yqgF*. To achieve *yqgF* regulation, we used a *S*. Typhimurium *yqgF*^{TD} (tetracycline dependent) and a *S*. Typhimurium P_{BAD}gtgRP$_{left}$-*yqgF* (negatively regulated by Arabinose). Using both constructs we found that YqgF is important for growth. Expression of *yqgF* was studied using both inducible system and we found that *yqgF* expression directly correlates with expression of *invI*, *orgB, avrA,* among others. We conclude YqgF could be acting on anti-termination of these genes. Currently we are studying the regulon of *yqgF* by massive sequencing, but also using a modified Tn5 transposon that produce transcriptional fusions to investigate the regulon of *yqgF*.

FONDECYT 11150588

CLP1-33

Study of non-coding RNAs in *Vibrio parahaemolyticus* strains

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A new form of genetic regulation in bacteria has been revealed with the identification of small non-coding RNAs (sRNAs). In *V. parahaemolyticus* there are 43 sRNAs identified but only RyhB and Spot 42 have been experimentally validated to date. Also, there is still a long way to go to understand the function of these molecules in different biological pathways, including virulence regulation. In this work, we analyzed the presence/absence of sRNA between pandemic and non-pandemic strains of *V. parahaemolyticus* from different origins. Additionally, we determine sRNAs differentially expressed when *V. parahaemolyticus* is growing in laboratory (isolation-condition; 37°C, 0.9% NaCl, and 0.04% bile salts) versus marine-like condition (12°C, 3% NaCl, and absence of bile salts) and sRNAs differentially expressed during infection within a mammalian host (data published by Livny et al, 2014) compared to the isolation condition. The RNA-seq results were corroborated through qRT-PCR during 4 hours of infection in Caco-2 cells. Our analysis showed that nine sRNAs were differentially expressed, six sRNAs increased their expression in isolation conditions (3 CsrB genes, 6S RNA, purine and lysine riboswitch) while three (Spot 42, RhyB and TPP Riboswith) decreased. Spot42 and RyhB sRNAs appear to be down-regulated under isolation conditions. On another hand, these small RNAs besides two ci-di-GMP II riboswitch, Qrr, Purine Riboswitch and RNA-OUT strongly increased their expression during infection. All data together suggest that these last sRNAs in *V. parahaemolyticus*are associated with virulence processes.

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CLP1-34

Genomic variations and underrepresented mutations in circulating influenza A viruses

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The influenza A virus (IAV) is a respiratory pathogen that circulates in humans yearly. Despite preventive measures and treatments, IAV is still a high morbidity pathogen. IAV have a high mutation rate which give the virus a mechanism for fast genomic evolution. Residue variations can be characterized as virulence markers. We analyzed the genomic variations of influenza genes of infected patients sampled in 2011 and 2013 in Chile. Nasopharyngeal swabs of 23 IAV RT-PCR-confirmed patients with A(H1N1)pdm09 IAV were obtained. At least 2 sequential samples were taken within 7 days after symptoms onset. Thirty-six haemagglutinin and forty-two neuraminidases were sequenced. The criteria for severe infection were established according to hospitalization parameters and/or derivation to Critical Care Unit after symptoms onset.Phylogenetic analysis revealed genetic variations and grouped the sequences into major clusters that generally corresponded to the year of collection, with the exception of divergent sequences belonging to patients, p1d1/2011, p15d1/2011 and 31673/2013. Serial samples grouped together, except for patients p3/2011, p21/2011, p13/2013, p20/2013 and p25/2013 in the haemagglutinin and p3/2011 and p12/2011 in neuraminidase segment. Five rare mutations were identified in functional regions of HA and NA, including four within antigenic sites of HA, which were further analyzed. Sequential samples from patient p25/2013 showed a K180Q change in HA. We identified a mixed genotype at this site early during infection, suggesting a plausible antigenic drift in HA within this individual, particularly since 180Q has become fixed in the current A(H1N1)pdm09 strain. Patient p3/2011 had a novel I223M mutation located within the catalytic pocket of NA. Deep sequencing identified a mixed population at this site during antiviral treatment. This reverted to the wild type genotype after treatment was finalized, suggesting that the 223M mutation could confer resistance to Oseltamavir. Our results demonstrate a high level of genomic variation of IAVs within and among infected individuals. This highlights the value of in depth and systematic analyses of viral genomes, which might allow elucidating relevant virulent markers during human infection.

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CLP1-35

A novel bioinformatics method to specifically identify Genomic Islands in the emerging pathogen *Klebsiella pneumoniae*

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Recently, the bacterial pathogen *Klebsiella pneumoniae* has attracted the attention of health authorities due to the appearance of hypervirulent and multi-resistant strains, which cause serious infections that are difficult to treat with the available antibiotics. The rapid emergence of those strains has been associated to the high genomic plasticity of this species, shaped by the acquisition of virulence and resistance determinants by horizontal gene transfer. Among the genetic elements involved in such plasticity are the genomic islands (GIs), which correspond to DNA fragments of variable length that can integrate or excise from the chromosome. GIs are preferentially inserted into genes encoding tRNAs and tmRNAs (tDNAs). Methods previously described for GI identification are mainly based on the examination of their sequence features, such as a GC content lower than chromosome average, as well as the presence of direct repeats or genes coding for integrases. However, few algorithms use genome disruption for predicting genomic islands. For the identification of GIs, the method described in this work compares every tDNA *locus* of a given genome against a manually curated *K. pneumoniae loci* database. The former corresponds to the tDNA loci in a context that have not been occupied by genomic islands, termed virgin *loci*. The new method compares each locus in the genome of interest with the correspondent virgin locus in the database, and GIs are predicted when a sequence larger than 10 kb interrupts the continuity of the unused locus. This novel algorithm also includes a new nomenclature for tDNAs and their context (proposed by our research group), as well as a module that allows the identification of direct repeats, improving the accuracy in defining the starting and ending position of a GI into the genome. Moreover, this method can identify GIs that have lost their direct repeats. Currently, this prediction tool is in its development stage, where Python, Biopython, Scipy and BLAST, among others, are being used to write the software.

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CLP1-36

Participation of the two component system ArcAB in the *Salmonella* Typhimurium resistance to Reactive Oxygen Species-related toxicity inside the neutrophil

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Salmonella Typhimurium (*S.* Typhimurium) is a pathogen that colonizes the intestine, and it is able to cause gastroenteritis in humans and systemic fever in the murine model. The immune response against this pathogen is initiated by the innate cells migration, including neutrophils, towards the infection focus, these cells are able to internalize the bacteria in the phagosomal compartment, where it is exposed to oxidative stress by the production of Reactive Oxygen Species (ROS) such as O^{-}_{2}, H_2O_2 and HClO. *Salmonella* has the ability to survive these conditions through gene modulation, allowing the pathogen to express detoxifying enzymes, virulence factors and it is even able to change its permeability towards the toxic compounds. There is evidence that ArcA in *S.* Typhimurium has an important role in the resistance to ROS, in our laboratory it has been proven *in vitro* through transcriptional analyses that ArcA modulates the bacterial gene response in aerobic conditions and in the presence of H_2O_2. In the same context, we have demonstrated that ArcA is required during the invasion of murine neutrophils and in the systemic infection in mice. Therefore, we hypothesize that the ArcAB two component system regulates the pathogen ability to whiststand the toxic compounds generated inside neutrophils. We measured the total ROS, O^{-}_{2} and H_2O_2 concentrations in the mutant strains lacking *arcA* and *arcB* separately and compared to the wild type strain levels and found that the toxicity inside the neutrophil is maintained inside the cell regardless of the infecting bacterial strain, additionally we found that some detoxifying enzymes as well as genes related to the maintenance of the redox state are regulated by ArcA in this conditions. We therefore postulate ArcA as a relevant contributor the the pathogen ability to endure the adverse conditions encountered inside neutrophils.

FONDECYT 1160315

CLP1-37

Intragastric administration of cigarette smoke condensate alters Paneth cells integrity and functionality in mice

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Background. Paneth cells are specialized epithelial cells of the small intestine. They contain multiple secretory granules, filled with antimicrobial peptides, which are essential for controlling the growth of microorganisms and maintaining intestinal homeostasis. Alterations in its function are associated with an imbalance of the normal microbiota and inflammatory processes, such as Crohn's disease (CD). Various studies have shown that cigarette smoke exposure, the main environmental risk factor for CD, affects the intestinal barrier of the small bowel, increasing intestinal bacterial translocation to the underlying lymphoid nodules. However, its effect on Paneth cells is unknown. **Objective**. To implement a mouse model of intragastric exposition to cigarette smoke condensate (CSC), and to evaluate its impact on Paneth cells (PCs) integrity and functionality. **Methods.** C57BL/6 male mice (n=4 per group) received 200µg of CSC, 400µg of CSC or vehicle intragastrically, 3 times a week for 2 weeks. After 2 weeks, ileum and colon samples were obtained for histopathological analysis. Also, ileum samples were used to evaluate PCs integrity with Alcian blue/PAS staining protocol, and to quantify the expression of antimicrobial peptides by qPCR. **Results.** Mice treated with 400ug of CSC exhibited signs of inflammation and distortion of the ileal, but not colonic,mucosal architecture.; lower number of PCs per crypt (p=0,0017); and reduced expression of cryptdin-1 (p=0,001), cryptdin-4 (p=0,001) and RegIIIγ (p=0,001) compared to the vehicle group. Mice treated with the lower dose of CSC only showed a reduced expression of RegIIIγ compared to the vehicle group. The distribution of PCs granules was not significantly different between the three groups. **Conclusion.** It was possible to implement a model of intragastric exposure to cigarette smoke in mice, which induces ileal inflammation. Moreover, our results show that the exposure to cigarette compounds alters Paneth cells integrity and functionality. This effect may promote gastrointestinal infections, or trigger inflammatory processes.

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CLP1-38

SOCS protein expression and its relationship with the innate immune response in obese infants

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Background. Viral respiratory infections are the leading cause of hospitalization in young children, especially by respiratory syncytial virus (RSV). Additionally, obesity has been associated with a worse clinical course in viral infection. In Chile, childhood obesity reaches 34%. This pathology is characterized by a low-grade chronic inflammatory state, due to increased production of pro-inflammatory cytokines, among them leptin. In adults, leptin has been reported to increase levels of proteins the suppressors of cytokine signaling (SOCS), which may potentially alter the production of antiviral proteins and interferons (IFNs), triggering a poor antiviral response. Thus, the objective of this study was to measure the expression of SOCS in obese children and its association with a lower antiviral response induced by lipopolysaccharide (LPS) in isolated peripheral blood mononuclear cells (PBMC). **Methods.** SOCS1 and SOCS3 mRNA levels were measured from PBMC of 3 obese children and 3 normal weight children, all under 2 years old. SOCS, IFN-γ and 2´,´5-oligoadenylate synthetase (OAS) antiviral response gene were quantified in PBMC cultures, in response to TLR4 stimulation with LPS (1 µg/ml). Also, PBMC of one normal weight child were stimulated with LPS (1 µg/ml) and leptin (100 ng/µl) and expression of OAS and quantification IFN-γ was analysed. mRNA of SOCS and OAS was evaluated by qRT-PCR. Leptin and IFN-γ was analysed in plasma and culture by Magnetic Luminex®assay. **Results.** Obese children had a higher SOCS3 expression ($p < 0.049$) than normal weight children, but not SOCS1. No significant differences were found in plasma leptin production (M 2.5 ng/mL, 1.0-6.3) or in the expression of SOCS1 and 3, OAS and IFN-γ in PBMC cultures of obese children compared to normal weight children. However, PBMC of normal weight child stimulated with LPS and leptin showed a decreased level of expression of OAS and IFN-γ. **Conclusion.** Obese children present higher levels of SOC3 protein expression in blood PBMC than normal weight children. However, upon LPS stimulation only of obese children PBMC, despite a trend, there was no significant decrease in IFN-γ and OAS gene expression, probably due to the lack of leptin in the medium.

FONDECYT 11150599

CLP1-39

The hydroxylation level of lipid A is critical for the interaction of *Salmonella* Enteritidis with murine macrophages and its ability to colonize the murine host

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Lipid A is the main bioactive component of lipopolysaccharide, acting as a strong inducer of the immune response. Because of this property, many pathogenic bacteria modify the structure of their lipid A in order to reduce its immunogenicity. Although several of these modifications have been fully characterized, hydroxylation of lipid A has not been studied in depth. In *Salmonella* Typhimurium, this modification is catalyzed by LpxO, and deletion of *lpxO* gene causes attenuation both *in vitro* and *in vivo*. In this work, we studied the influence of lipid A hydroxylation on the interaction of *Salmonella* Enteritidis with murine macrophages *in vitro* and its virulence *in vivo*. To this end, we constructed a Δ*lpxO* mutant of *S*. Enteritidis NCTC13349 by allelic replacement and then we generated the corresponding *trans*-complemented strain. The hydroxylation of lipid A for each strain was evaluated by mass spectrometry. In addition, the invasion and intracellular survival of each strain was assessed in RAW264.7 murine macrophages, and their virulence *in vivo* was evaluated by oral infections of C57BL/6 mice. According to our results, the Δ*lpxO* mutant produced a non-hydroxylated lipid A, and displayed an increased invasion and a reduced intracellular survival in RAW264.7 macrophages (especially after long periods of infection) in comparison to the wild-type strain. In contrast, the *trans*-complemented mutant produced a fully hydroxylated lipid A, and exhibited a partial restoration of the wild-type invasive phenotype, but keeping the intracellular survival defect showed by the Δ*lpxO* mutant. In orally inoculated mice, the bacterial load of the Δ*lpxO* mutant recovered from the intestine and internal organs (spleen and liver) was lower as compared with the wild-type strain. Of note, the lowest bacterial load was recovered when mice were infected with the *trans*-complemented mutant, which only produced fully hydroxylated lipid A. Overall, our results suggest that an adequate level of lipid A hydroxylation is required for *S*. Enteritidis to escape phagocytosis and to survive intracellularly in murine macrophages, promoting the intestinal and systemic colonization of the murine host.

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CLP1-40

Piscine Orthoreovirus (PRV) detected in Chilean Rainbow trout is related to PRV strains from Norway and Canada

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The Piscine orthoreovirus (PRV), a new member of the *Reoviridae* family, is associated with heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon. The HSMI is a highly prevalent disease first diagnosed in Norway in 1999, where it is considered one of the most commercially important diseases of salmon farming. In Chile, the epidemiology or prevalence of PRV in Atlantic salmon or rainbow trout have not been deeply studied, however it has only been reported mortalities associated to HSMI in Atlantic salmon. In 2011, farmed rainbow trout mortalities with clinical signs and histopathological findings similar to those described in Atlantic salmon HSMI-diseased were reported; this novel illness was known as Idiopathic Syndrome of Rainbow Trout (ISRT), and actually no marine pathogen has been linked to this new disease. We analyzed heart tissue samples of rainbow trout mortalities related to ISRT (from one fish hatchery located in southern Chile) for PRV detection using RT-qPCR assay, and conventional RT-PCR was used to amplify the complete sequence of PRV segment S1. Low-to-mild viral load and high prevalence of PRV was found in farmed rainbow trout clinically diagnosed with ISRT. Phylogenetic analysis was performed with the sequences obtained to establish their relationship to PRV strains from another countries. The PRV S1 segment sequences found in rainbow trout with ISRT are phylogenetically related to PRV strains grouped in sub-genotype Ia (Norway – Canada) instead of sub-genotype 1b (Norway – Chile), as previously described. This study is at our knowledge, the first description of PRV in farmed rainbow trout associated with a pathological condition, which is comparable to HSMI in Atlantic salmon, although more studies are needed to establish a direct linkage between PRV with ISRT.

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CLP1-41

Effect of sucrose in the expression of virulence genes of *Streptococcus mutans* and *Streptococcus sanguinis* grown in dual-species biofilm

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Background. As part of the dental biofilm, *Streptococcus mutans* (Sm) synthesizes three glucosyltransferases enzymes (GtfB, -C and -D; encoded by the *gtfB*, *gtfC* and *gtfD* genes) associated with the formation of extra and intracellular polysaccharides (glucans) from sucrose. These enzymes allow bacterial adhesion to enamel creating conditions for a cariogenic biofilm. As part of the commensal dental biofilm, *Streptococcus sanguinis* (Ss) has been typically isolated from caries-free children and adults. *S. sanguinis* produces only one glucosyltransferase (GtfS; encoded by the *gtfP* gene) and H_2O_2, by pyruvate oxidase (SpxB; encoded by *spxB* gene). H_2O_2 is an antibacterial compound, interfering with *S. mutans* tooth colonization. How both bacteria express these virulence genes upon sucrose exposure has not been described. A caries model with dual-species biofilms was used to establish the effect of a cariogenic challenge with sucrose on the expression of competition-related genes. **Methods.** Dual-species biofilms of Ss SK36 and Sm UA159 under different colonization sequences of the dental enamel were assayed on bovine enamel slabs and exposed to 10% sucrose for 5 min, 3x/day for 5 days. Biofilms exposed to 0.9% NaCl were used as negative caries-control. Biofilms were recovered from the enamel slabs and total RNA was isolated. Transcriptional analysis of *gtf*s and *spxB* genes was performed. Ct-values were normalized for the control gene (16S rRNA) and subsequently expressed as function of the caries-negative condition. Wilcoxon test was used to compare the expression of the genes, relative to the control. **Results.** The Sm *gtfB* gene was downregulated in simultaneous Sm and Ss colonization (Sm=Ss), whereas upregulation was detected when Ss was the first colonizer (Ss-Sm) ($p<0.05$). Both the *gtfC* and *gtfD* genes were upregulated in the Ss-Sm condition. Eleven-fold upregulation of the *gtfP* gene was observed in biofilms where Sm was the first colonizer (Sm-Ss) ($p<0.05$). A moderate overexpression of the *spxB* gene in the Sm-Ss condition versus control was observed. **Conclusion.** This study suggests that under a demanding sucrose-mediated condition, both Ss and Sm require to overexpress their virulent traits to effectively compete in the dental biofilm, especially when the competitor has previously colonized the ecological niche.

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CLP1-42

Antiviral role of Heme Oxygenase-1 enzyme on the Pneumovirinae viruses hRSV and hMPV

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Background. Human respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV) are two major respiratory pathogens that can cause severe respiratory diseases in infants, young children and the elderly. Treatment of hRSV and hMPV infections is only supportive and includes supplemental O_2 and hydration. However, efficient vaccines or antiviral drugs are not available. Here, we examined the effects of inducing the activity of the host enzyme heme oxygenase-1 (HO-1) as a possible antiviral strategy against these two pathogens. **Methods.** A549 cells were infected with hRSV or hMPV in the presence or absence of CoPP (an HO-1 inductor), SnPP (an HO-1 inhibitor), or vehicle control for 24 h and then cells were analyzed to measure infection. For in vivo experiments, BALB/cJ mice were treated for 24 h with CoPP, SnPP or vehicle control and then intranasally inoculated with either mock or hRSV. Disease progression was monitored by animal weight loss after infection with each pathogen. Mice were euthanized and BALFs analyzed for neutrophil infiltration by flow cytometry and lung homogenates of each experimental group of infected mice, were collected and quantified for viral loads. The same methodology was applied to transgenic mice (tTA-HO-1) overexpressing human HO-1 in in MHC-II$^+$ cells. **Results.** We observed that HO-1 induction can reduce airway epithelial cell permissivity to both hRSV and hMPV infection. Reducing viral replication and viral particle production. Also, HO-1 induction reduced the disease parameters induced by hRSV in infected mice. Further, HO-1 induction also decreased viral replication and lung inflammation, as evidenced by a significant reduction in neutrophil infiltration to the airways. Furthermore, similar antiviral and protective effects were observed by inducing the transgenic expression of human HO-1 in MHC-II+ cells in mice. Finally, in vitro data suggest that HO-1 induction can reduce airway epithelial cell permissivity to the virus. **Conclusion.** HO-1 induction decreases the infection of hRSV and hMPV in epithelial cells and could protect the host from the pulmonary pathology developed upon hRSV infection.

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CLP1-43

MarT down regulates STY1408 expression, a new gene involved in virulence of *Salmonella*

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Salmonella enterica serovar Typhimurium (STM) is considered a generalist pathogen since it infects birds, mice and humans. On the other hand, *Salmonella enterica* serovar Typhi (STY) is considered a restricted host pathogen that only infects humans. Despite these differences, STM and STY share a 97% of identity between their shared genes. The horizontal transfer and the loss of genetic material have been essentials for differentiation of both serovars. The genomic island SPI-3 is an example that arose as a product of these two processes. SPI-3 encodes for *misL* and *marT*. In STM, *misL* is required for gut colonization of birds and mouse, orally infected. Furthermore, it has been reported that MarT positively regulates *misL* in STM, in a direct manner by recognizing the consensus sequence TNAAANNNNNTNAAA on its promoter. On the other hand, SPI-3 of STY contain ORFs that are found as pseudogenes (*marT* is one of these); while others ORFs, as is the case for *surV* (that is involved in the H_2O_2 resistance) is only present in STY. Interestingly, the heterologous expression of $marT_{STM}$ in STY negatively regulated *surV*, at the level of transcription. In addition, transcriptomic analysis of STM inside of macrophage shows that *marT* expression increase 10 folds, reinforcing the idea that this gene is involved in the virulence of STM. For these reasons, the objective of this work is to find and characterize new genes regulated by *MarT*. With this purpose, we did a global *in silico* search of the TNAAANNNNNTNAAA sequence to identify possible MarT targets. One gene negatively regulated by MarT identified and validated by qRT-PCR was STY1408, a gene homologous to chemoreceptors in the MCP family. Semisolid plate assays shown that STY1408 is involved in motility of STM and STY. Infection of HEp-2 cells with STM and STY mutants in STY1408 gene shown a defect in invasion. Oral infection of mice with STY1408 null STM are defective to colonize liver and spleen. We conclude that MarT negatively regulates STY1408 gene involved in the virulence of *Salmonella*.

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CLP1-44

Distinctive gut microbiota is associated with diarrheagenic *Escherichia coli* infections in Chilean Children

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Background. Diarrheagenic *Escherichia coli* (DEC) strains are a major cause of diarrhea in children under 5 years of age. DEC pathogenicity relies on the interaction of bacteria with the host's resident gut microbiota. Identification of resident indicative bacterial taxa in DEC-positive diarrhea stool samples of children and its effect on DEC adherence, cell pro-inflammatory response may be fundamental for understanding microbiota's role in DEC pathogenicity. **Methods**. We analyzed 63 diarrheal stool samples from Chilean children 1-5 years of age; 32 positives for at least one DEC pathotype (DEC group) and 31 of those caused by enteric viruses as sole pathogen (Viral group). In addition, 30 stool samples from healthy children, negative for enteric pathogens were analyzed (Healthy group). The 16S rRNA gene was amplified and sequenced using 454-pyrosequencing. Sequences were clustered into operational taxonomic units (OTUs) and their representatives were used to assign them to operational phylogenetic units (OPUs) in order to define DEC microbiota and indicative species. Effect of *Citrobacter werkmanii* on DEC adherence and induction of IL-8 secretion on T84 cells was evaluated. **Results**. An increase in sequences belonging to the phylum *Proteobacteria* in the DEC group compared to the viral and healthy groups was observed. Samples displayed a statistically different community structure by sample grouping by redundancy analysis and ANOVA. *Citrobacter werkmanii* (p=0.0012) *Escherichia albertii* (p=0.001), *Haemophilus parainfluenzae* (p=0.003), *Granulicatella elegans* (p=0.019), *Yersinia enterocolitica* (p=0.032) *and Haemophilus sputorum* (p=0.036) were found as the main indicative species for the DEC group when compared to the viral and healthy groups. DEC growth decreased when it was co-cultivated with *Citrobacter werkmanii*; also it was observed that DEC adherence on T84 cells was decreased with no changes in the induction of IL-8 secretion by infected epithelial cells. **Conclusion**. We identified *Citrobacter werkmanii* as one indicator species of the DEC group. Our results showed that *Citrobacter werkmanii* may be relevant in understanding of the relationship between resident gut microbiota and DEC.

FONDECYT 1160426

CLP1-45

Salmonella Typhimurium proteins PphA and SteC play a role in the manipulation of autophagy in the amoeba *Dictyostelium discoideum*

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Recently, we demonstrated that *Salmonella enterica* serovar Typhimurium is able to survive intracellularly in the amoeba *Dictyostelium discoideum*. Although the molecular mechanisms involved in this process have not been characterized, it has been reported that *D. discoideum* uses autophagy as a defense mechanism against intracellular pathogens such as *Salmonella*. In this work, we aimed to identify a possible role for proteins PphA and SteC in the manipulation of autophagy in *D. discoideum*. PphA is a hypothetical serine/threonine phosphatase, and SteC is a serine/threonine kinase that is secreted through the type 3 secretion system encoded in pathogenicity island SPI-2. First, we evaluated the expression levels of *pphA* gene by measuring the β-galatosidase activity of a *S*. Typhimurium strain harboring the promoter of *pphA* fused to the *lacZ* reporter. In addition, we assesed the production of this protein by western blot using a strain of *S*. Typhimurium expressing a fusion of PphA to the 3xFLAG epitope. Our results indicate that *pphA* is actively transcribed when *S*. Typhimurium is grown at 37°C in rich medium (LB broth). In addition, under the same culture conditions the PphA-3xFLAG fusion protein is immunodetected. Next, we performed infection assays in *D. discoideum* cells expressing the autophagosome marker GFP-Atg8, using the wild-type strain of *S*. Typhimurium and mutant strains Δ*pphA* and Δ*steC*. The infections were analyzed by confocal fluorescence microscopy to quantify the amount of autophagosomes generated. We observed that amoebas infected with the mutant strains Δ*pphA* and Δ*steC* present less autophagosomes than amoebas infected with the wild-type strain. Taken together, our results indicate that PphA protein is expressed and produced by *Salmonella*, and suggest that both PphA and SteC are involved in the manipulation of autophagy in *D. discoideum*.

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CLP1-46

Herpes Simplex Virus type 1 neuronal infection alters synaptic plasticity processes through Arc protein dysregulation

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A rapid response to external stimulus during synapsis is mediated by the expression of Immediate Early Genes (IEGs). One relevant member of these genes is the activity-regulated cytoskeleton-associated protein: Arc (also named Arg3.1) that plays a crucial role in the homeostasis of the synaptic plasticity and memory formation. Arc execute its function through a regulatory role in actin cytoskeleton and endocytosis of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (also known as AMPA receptors). Interestingly anomalously high levels of Arc have been found in patients with Alzheimer Disease (AD), a neurodegenerative pathology directly linked with Herpes Simplex Virus type 1 (HSV-1) infection. To date, no studies have evaluated whether persistent neurotropic HSV-1 infection modulates expression and function of Arc protein in neurons. Here we report that HSV-1 neuronal infection in *in vitro* and *in vivo* models significantly increases Arc expression. In addition, we found that Arc is distributed distinctly from control cells, showing a robust perinuclear distribution by immunofluorescence analysis. Moreover, we found that HSV-1 infection caused alteration in neuritic processes related to cytoskeleton, increasing the number of filopodia-type protrusions, which are known to be immature dendritic spines. However, functionally these infected neurons presents a decrease in glutamatergic response compared to mock infected neurons, as we see in the calcium imaging assays. Altogether, these results suggest that HSV-1 infection affects normal synaptic processes in neurons in an Arc dependent manner, which could have consequences on both, synaptic homeostasis and viral spread.

FONDECYT 1150574

CLP1-47

Phenotypic diversity in biofilm produced by *Salmonella enterica* isolates from different host in Chile

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During the past 20 years *Salmonella enterica* has been a major cause of foodborne infections (ETAs) in humans. In the Chilean population, Salmonellosis is an endemic disease and according to the Institute of Public Health (ISP), the current infection prevalence rates are twice from those recorded 10 years ago. The serovar with the highest importance is *Salmonella* enterica serovar Enteritidis (*S*. Enteritidis), being responsible of more than 65% of the cases that occur in the country. The persistence of the bacterium in different ecological niches depends on its ability to resist or survive both favorable and unfavorable conditions for growth. The ability to form biofilms is crucial for the persistence of bacteria in adverse conditions. In these aggregations, bacteria are protected by the layer of exopolysaccharides surrounding them, which increases survival under stressful conditions and favors persistence within the host. The main goal of this work was to characterize 83 Chilean *S. enterica* isolates belonging to different serotypes, hosts and geographical regions of Chile in their ability to form biofilms at 28°C and 37°C, using the colorimetric method of plaque titration. The morphotype of the colonies obtained in congo red and calcofluor white plaques was used to determine the kind of exopolysaccharide produced by these strains. In this characterization, 81% of *S. enterica* isolates formed biofilm but showed high qualitative variability. This ability was not associated to either the serotype or the bacterial host. Among the biofilm forming strains of *S.* Enteritidis, 83% of them produced cellulose and curly, structures implicated in the adherence to epithelial cells, glass and plastic surfaces. However, most of *S.* Agona, *S.* Havana, *S.* Infantis and *S.* Heidelberg isolates produced curly but not cellulose, even though are good biofilm formers. These results suggest that cellulose is not a fundamental component for biofilm formation in *S. enterica*.

Proyecto Interno Universidad Autonoma de Chile DPI 61-2015 and FONIS SA15I20094

CLP1-48

Detection and identification by PCR of *Brucella* gender bacteria in peri-domicilary water samples of urban and rural houses of the Region de Los Ríos, Chile

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Brucellosis is a zoonotic disease produced by bacteria of the genus *Brucella*. In humans, it can produce from fever to systemic infections while in animals it commonly generates abortions. Worldwide it has been sought to eradicate this disease, because of its impact on public health and the great economic losses that it produces in the cattle industry. In Chile, a campaign for the eradication of bovine brucellosis has been carried out through the elimination of infected animals and vaccination; however, sporadic cases of brucellosis appear in flocks free of infection, without being able to establish the origin of the disease, which leads to look for other likely sources of infection, such as water. Due to the epidemiological importance of this bacterium, in this work we tried to detect genome of *Brucella* spp. in various water sources obtained from the peri-domiciliary environment in different areas of Los Rios Region. For this, by means of bibliographic search, *in silico* analysis and PCR assays the gene with the best *Brucella* spp. detection potential was determined, in order to perform conventional PCR on samples. In addition, to verify that the PCR positive samples corresponded to the genome of *Brucella* spp. a Sanger sequencing was performed to two samples. The results revealed that the IS711 gene is the most suitable for this study compared to the other genes analyzed (16S rRNA, OMP2 and BSCP31). Of a total of 899 DNA samples obtained from different peri-domiciliary water sources, 87 were PCR positive for *Brucella* spp. where the highest proportion was found in the Urban area (17.7%) and the lowest in the Rural Concentrated area (4.3%), both associated with a risk and protection factor respectively. We conclude that the IS711 gene together with its primers and their use by PCR proved to be effective for genome detection of *Brucella* spp., which was corroborated by Sangers sequencing. Finally, further studies are required that examine the real importance of water as a probable source of infection, determining that *Brucella* species are present and their possible role.

Dirección de Investigación de la Universidad Austral de Chile

CLP1-49

PCR real time as a tool for the evaluation of chemotherapeutic efficacy in chronic chagas disease

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Chagas disease (CHd) is a parasitic zoonosis caused by the flagellate protozoan *Trypanosoma cruzi* and a major public health problem in Chile and Latin America. The only trypanocidal drugs authorized for its treatment are nifurtimox (NFX) and benznidazole. With the application of Real Time PCR (qPCR) in the parasitological follow-up of the treated patients, it has been possible to obtain quantitative information on parasitic loads, fundamental aspect in the evaluation of the treatment of CHd. To evaluate the DNA extraction process of blood samples by X12 detection (Chromosome 12) and quantification of human DNA and, to determine the parasite load of *T.cruzi* in blood samples from individuals with chronic CHd treated with NFX in prolonged follow-up (average period of 6.6 years) by SYBR® Green detection system. DNA integrity was evaluated by determining the concentration of total DNA and amplification of a region of human chromosome 12 (X12). In addition, the parasite load of 21 samples of individuals under pre and post-therapy with NFX, was determined with two nuclear primers for *T.cruzi* (cruzi 1-cruzi 2). All samples were positive for qPCR-X12, with DNA concentration ranges from 0.133 to 7.18 ng/µL in pre and post-therapy. We also evaluated 32 samples of seronegative individuals for CHd, whose range of genomic DNA concentration was of 0.021 to 10.9 ng/µL. The parasite load of *T.cruzi* determined by qPCR under condition of pre-therapy for the 21 cases ranged from 2.4 to >100.000 par.eq/mL. In post-therapy conditions, only 5 patients were positive, with loads ranging from The qPCR technique is an alternative tool to detect and quantify *T.cruzi* directly in the peripheral blood on individuals with chronic CHd, as well to evaluate X12 as an exogenous internal control. A reduction in the parasitic load of *T.cruzi* was observed when comparing the pre and post-therapy conditions, which allows us to infer pharmacological efficacy in the treatment with NFX with higher percentages to those described in literature.

Proyecto FONDECYT Regular 1161485

CLP1-50

Development of a rapid and efficient detection method for Infectious Salmon Anemia Virus using Reverse Transcription - Loop mediated Isothermal Amplification (RT-LAMP) coupled to a colorimetric assay

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ISA virus (ISAv), is the etiologic agent of infectious salmon anemia, belongs to the family Orthomyxoviridae, being an enveloped virus with eight single-stranded negative-sense RNA segments. This pathogen mainly affects *Salmo salar*, producing important losses in farming on Europe, North America and Chile. In this country, SERNAPESCA, generate sanitary programs of vigilance and control of ISAv, which require monitoring constantly zones of infection. The current detection methods are varied, include RT-qPCR, with SYBR and Taqman chemistries, Monoclonal antibodies, among others; however, these techniques use complex and expensive laboratory equipment and highly specialized personnel, without mention the long time required. The loop mediated isothermal amplification technique, consist in the use of several sets of primers, two of which will generate loops at the ends of the amplicon allowing, the production of secondary structures that, when replicated continuously in cascade, will form new structures of various sizes. The reaction is catalyzed by a specially engineered DNA polymerase, which under specific conditions displays retrotranscriptase activity. In order to develop an RT-LAMP assay for ISAv, we obtained a consensus sequence, according to OIE recommendations, from epizootic strains and by analysis on databases. Of all primers sets were designed with an appropriate software and finally one of them was selected to experimental assays. The amplification conditions were optimized, aiming for high sensitivity and specificity. All experimental validations were done using standard and field samples. Finally, the reaction was successfully coupled to a colorimetric assay for a rapid detection of results. Optimal amplification results were obtained at 60 °C, in a time of 30 minutes with retrotanscriptase activity, without the need of another enzyme associated to the assay, with an estimated detection limit of 10^2 copies of the target genetic material. In conclusion, this method of detection has shown to be fast, specific and sensitive as well as inexpensive. Furthermore, when coupled to a colorimetric reaction, it only requires the use of a steady temperature to obtain fast results of easy interpretation.

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CLP1-51

Detection of key bacterial pathogens responsible for human ocular infectious keratitis by multiplex qPCR with fluorescent probes

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One of the main causes of irreversible blindness in the world is infectious keratitis. It generates a corneal ulcer, which threatens vision. The most common causative microorganisms involved in infectious keratitis are from bacterial origin, namely *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus epidermidis*. Generally, the diagnosis is performed by clinical observation, and the techniques used for pathogen identification are traditional biochemical tests. The current procedure against suspected infectious keratitis is a broad-spectrum antimicrobial therapy which brings multiple difficulties associated with the use of non-specific antimicrobials. At the present and taking advantage of new technologies, the most efficient manner to identify bacterial pathogens is through molecular biology strategies such as multiplex qPCR. Therefore, in this work we aimed to develop a new detection technique for these three main bacterial pathogens, using a multiplex qPCR with fluorescent probes to provide a more rapid, specific, sensitive and quantifiable diagnosis. For this, the coagulase gene (GenBank Gene ID: 31213026) from *S. aureus*, the methylated-DNA-protein-cysteine S-methyltransferase (GenBank, Gene ID: 1056095) from *S. epidermidis* and the *oprD* porin D (GenBank Gene ID: 881970) from *Pseudomonas aeruginosa* were chosen as specific genes to identify each species in a complex sample. Then, specific primers for each gene were designed and used for their amplification from bacterial gDNA by PCR. Each PCR product was gel purified and cloned in the pCR2.1 cloning vector. After confirmation of cloned fragments by sequencing, specific primers for multiplex qPCR were designed, including 1 probe for each gene. For more accurate quantification of bacterial pathogens in a sample, the plasmid DNAs for all three targets were used for absolute quantification. Currently, important qPCR experimental details and information that is critical to performance and reliability of results are being investigated. For instance, we established that each primer pair and probe are specific for each gene using a pool of plasmid DNAs as template. Now, tests on multiplex qPCR conditions using donor samples are under standardization. We conclude that this species-specific multiplex qPCR method will provide a more sensitive tool for identification of species involved in infectious keratitis, with future posttranslational applications.

Grupo de marcadores inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso

CLP1-52

Identification of enteropathogenic bacteria isolated from produce farms and irrigation waters in the Metropolitan Region, Chile

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In recent years, the vegetables have become more relevant in the consumption of the population. Therefore, the presence of animal feces and the contamination of surface water intended for irrigation in farms are sources for the existence of pathogenic microorganisms in agricultural production. Some pathogens isolated from vegetables are *Salmonella* sp. and *Campylobacter* spp., which have been associated with important foodborne diseases outbreaks. Previous studies in five places in the Metropolitan Region (MR) in Chile have showed the presence of strains of *Salmonella* spp. which are the same clonal type with the human strains detected in clinical. The main objective of this study is to identify the presence of *Salmonella* spp. and *Campylobacter* spp. in five points where agricultural production is performed, considering samples of vegetables, soils and irrigation water. The collection points in this study were planned considering the previous points in the past study. These points were areas of 400 to 600m^2 each one. The places were Isla de Maipo, María Pinto, Melipilla, Peñaflor and Talagante. For each point was considered to have close agricultural production farming and irrigation water flow. A total of 1524 samples were carried out between May 2016 and April 2017 (1270 vegetables, 230 soil and 24 irrigation water). The vegetables analysed correspond to lettuce, beans, alfalfa, celery, oats, chard, broccoli, potatoes and soybeans. The detection of *Salmonella* sp. was carried out by miniaturized most probable number technique based on ISO 6579-2. *Campylobacter* spp. was performed by BAM-FDA online methodology. A total of 3 samples from soil and 1 sample from irrigation water were positive. The genus detected were *Salmonella* Infantis and *Salmonella* Muenchen, respectively. A low detection 3/230 and 1/24 (soil and water respectively) could be explained by the numerous and diverse microorganisms present in the analysed samples and their complex analysis. Therefore, the presence of these microorganisms, the pathogenic potential and their transmission way are a potential risk to the health of the population. The strengthen sanitary control in the production of vegetables in the Metropolitan Region is still necessary.

Proyecto FONIS SA15I20094

CLP1-53

Genotypification of spoilage poultry *Pseudomonas* by RAPD as a simple tool to indirectly identify biofilms in the processing line

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Background. Biofilms in poultry industry can be associated to pathogenic and/or spoilage bacteria, among them *Salmonella, Campylobacter* or *Pseudomonas.* These bacteria are a major source of enteric diseases or major spoilage agents that reduce safety and shelf life of poultry products. The globalization of Chilean poultry exportations require lower bacterial loads. To evaluate the presence of *Pseudomonas* biofilms we can use a simple molecular methodology like Random amplification of polymorphic DNA (RAPD). DNA fingerprinting allow us to identify biofilms showing a similar pattern in bacteria from different poultry samples. The aim of this work was to characterize *Pseudomonas* isolates from poultry samples with different production dates by RAPD, and compare the resulting DNA fingerprint as an indicator of bacterial biofilms in the processing line. **Methods.** 24 isolates of *P. fragi* and 14 *P. fluorescens* from 11 poultry samples were obtained from a single industry, during different production dates and sampling time. Isolates were characterized through RAPD using a 10 mer primer (5`-AGCGGGCCAA-3`) and an annealing temperature of 36 ºC. All *Pseudomonas* isolates were grouped into RAPD types which were considered similar (≥85% Dice similarity) based on DNA patterns. **Results.** 24 *P. fragi* isolates were grouped into 13 RAPD types, from which 8 included two or more isolates obtained in different sampling times. 14 *P. fluorescens* isolates generated 12 RAPD types and only 2 isolates from different poultry samples had the same RAPD types. **Conclusion.** The presence of *Pseudomonas* isolates with similar RAPD types from different sampling times, evidences the presence of persistent biofilms in one or more steps during the poultry processing line. The results suggest that *P. fragi* has greater ability than *P. fluorescens* to persist in biofilms. Each enterprises require more research to locate the biofilms and to apply a BPM solution.

CONYCIT - PhD scholarship Number 21120298

POSTER SESSION II

CLP2-1

Assessing the metabolic profile of marine streptomycetes from Chilean coasts

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The alarming increase in bacterial resistance to commonly used drugs has made urgent the need for antibiotics discovery. The *phylum Actinobacteria* represents a prominent group of microorganisms for the production of bioactive compounds. Actinobacteria has contributed to nearly 40% of the bioactive secondary metabolite production, where nearly 80% of those are produced by the genus *Streptomyces*. The decrease of the number of bioactive compounds from traditional sources such as soil have made critical the investigation of microorganisms from underexplored habitats as a source of novel therapeutic agents, such as the oceans. Marine sediments and sponges are nutrient rich habitats, harbouring a considerable bacterial biodiversity with metabolic and genetic potential to develop bioactive secondary metabolites. The coast of Chile is a favourable ecosystem for this purpose, comprising an extensive coast with a plurality of climates and landscapes. This study focuses on streptomycetes isolated from marine sediments, sponges and a sea urchin from unexplored regions of both continental and insular Chile ranging from the III to the XI Region, including Easter Island. Various culture conditions were used and 31 streptomycete strains were isolated. Furthermore, these strains were studied to establish their antibacterial activities against five model strains: *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa*, demonstrating abilities to inhibit bacterial growth of Gram-positive bacteria. Chemical dereplication of streptomycete strains through LC-HRMS analysis permitted the evaluation of crude extracts derived from our collection for the antibiotic production potential. By combining various criteria such as phylogenetic designations, bioactivity screening, metabolic profiling and dereplication of selected streptomycete strains, we were able to pinpoint 3 strains for further scaling-up studies, suggesting that bioactive compounds produced by these strains could have possibilities for being novel.

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CLP2-2

Synthesis of metal-nanostructures exhibiting antibacterial properties by metal-reducing bacteria

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Given their wide range of applicability, there is a growing demand for nanostructured compounds mainly, as antibacterial agents. Many different microorganisms as well as metal-reducing enzymes can synthesize metal-containing nanostructures (NS). In particular, enzymes involved in metal tolerance belong to the family of flavoproteins which reduce efficiently metals and/or metalloids to their elemental state generating insoluble, less toxic, metal(loid)-containing NS. NS synthesis has been observed in the absence of oxygen, a condition which prevents the formation of reactive oxygen species and oxidation of the metal, resulting in larger NS exhibiting more regular shapes. The search for new, environmentally safe and more economical methods has made biological systems attractive candidates for both *in vivo* and *in vitro* NS synthesis. In this study, NS were generated *in vivo* using *Acinetobacter schindleri* and *Staphylococcus sciuri* and *in vitro* by crude extracts of these strains and *Escherichia coli* overexpressing the flavoprotein glutathione reductase (GorA). NS were synthesized aerobically and anaerobically, and their morphology, size and composition characterized using transmission electron microscopy, dynamic light scattering and X-ray emission spectroscopy, respectively. It was evaluated whether these NS exhibit antibacterial properties against *E. coli* BW25113. SeNS showed a spherical morphology and did not inhibit *E. coli* growth. On the other hand, TeNS exhibited an elongated and circular morphology and those synthesized aerobically were smaller in size as compared to those generated anaerobically. While TeNS generated anaerobically using crude extracts of anaerobically-grown *E. coli* (overexpressing GorA) slow down growth, those generated aerobically inhibited completely *E. coli* growth. The observed difference could be due to the smaller size of the aerobically-synthesized TeNS, since they present greater surface area associated to a faster speed of release of the metalloid than the larger ones. In this context, obtaining NS with antibacterial properties opens up the possibility to use it in biomedicine as possible agents against pathogenic strains resistant to existing bactericides.

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CLP2-3

Regulation of Tellurite-induced stress granules during HIV-1 infection

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Following a viral infection, the cell is able to mount an anti-viral response, reprogramming the translation of cellular messenger RNAs to create an unfavorable environment for virus replication. Translation stopping induces the formation of Stress Granules (SGs), which are dynamic cytoplasmic structures enriched in heterogenous messenger RNAs, translation initiation factors and 40S ribosomal subunits. The assembly of SGs can be induced by drugs such as Selenite and Arsenite, which produce oxidative stress in the cell, however, it has been described that Human Immunodeficiency Virus type I (HIV-1), a member of the Retroviridae family and the etiologic agent of the Acquired Immunodeficiency Syndrome (AIDS), has developed mechanisms that allow it to block the formation of SGs to promote viral replication. Tellurite (TeO_3^{-2}), is a highly toxic compound for many organisms whose presence in the environment has increased considerably due to its use in industrial processes and has been associated with potential adverse effects on public health. It has been reported that its cellular toxicity is mediated by oxidative stress, whereby its effect on the formation of SGs at different concentrations and times of treatment was evaluated and its effect on HIV-1 replication was studied using U2OS osteosarcoma cells stably expressing the protein associated with SGs, G3BP-1 fused to GFP and U2OS cells expressing Gag-RFP viral protein. In this work we show that Tellurite is able to induce the formation of nuclear aggregations of G3BP1 in U2OS cells. In addition, it was observed that HIV-1 expression results in the disassembly of such aggregations concomitantly to the formation of small nucleations of the viral Gag protein. These results suggest that the assemblage of SG is an important antiviral defense that HIV-1 has been able to intervene through various mechanisms.

FONDECYT 11140502

CLP2-4

Characterization of antibiotic resistance in *Salmonella* isolated from irrigation water and soil samples from the Metropolitan Region, Chile

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Background. *Salmonella enterica* is a foodborne pathogen that produces salmonellosis. Outbreaks are associated with consumption of food of animal origin, but in recent years around the world, outbreaks have been observed from consumption of raw vegetables, which can contaminate from water or soil. If strains also show resistance to antibiotics, they may represent a serious public health problem. The purpose of this study was to evaluate the susceptibility to antibiotics in strains of *Salmonella* spp., isolated from environmental sources such as rivers and soil. **Methods.** Samples were taken every two months along one year in six different points. The samples corresponded to superficial irrigation water and from soil where leafy vegetables are grown, within a radius of five kilometers from the irrigation water. The samples of soil were analyzed using ISO 6579-2 for *Salmonella*, and water samples were analyzed by ultrafiltration (Hill et al., 2005). The strains were serotyped by the Kauffman-White protocol. The susceptibility to antimicrobial was performed using Kirby Bauer (CLSI, 2015). **Results.** From 25 superficial irrigation water samples, a multi-drug resistant *S*. München strain was detected in one of them, with the following resistance phenotypes: gentamicin, tetracycline, sulfamethoxazole + trimethoprim, ceftiofur, ampicillin, cefadroxil, chloramphenicol, amikacin, ceftriaxone, kanamycin, nalidix acid. From the 250 soil samples, two strains were detected, one chloramphenicol resistant *S*. Infantis strain and one pansusceptible *S*. Infantis strain. **Conclusion.** Three *Salmonella* strains were isolated from soil and water samples. Two strains have antimicrobial resistance phenotypes, with that isolated from water being resistant to 11 of the 16 antibiotics analyzed. The potential use of soil and water for agricultural activities, suggest that environmental *Salmonella* strains represent a sanitary risk for public health.

FONIS PROJECT SA 15I20094

CLP2-5

Importation surveillance of phenicols, quinolones and tetracyclines for use in human and veterinary medicine in Chile from 1998 to 2015

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Many studies demonstrate that selection in the environment and transfer of resistance among bacteria occurs, especially in ecosystems impacted by use of antimicrobials in human activities. Information about resistant bacteria are obtained mainly throughout microbiological surveillance networks intended for human medicine (HM). However, this surveillance barely exists in veterinary medicine (VM). Information regarding the amounts of antimicrobials used is required to reveal their impact in selecting antimicrobial resistance, in a determined geographical area. Determination of the volume of medically important antimicrobials used in animal production and its comparison with use in HM is crucial to assess their relevance in generating antimicrobial resistance of clinical relevance. For this reason, we retrospectively investigated importation of phenicols, quinolones, and tetracyclines used for HM and VM in Chile between 1998 and 2015, with the aim of comparing the volumes destined for HM and VM. The antimicrobials imported were determined by using databases of the Central Bank of Chile, the Santiago Chamber of Commerce (1998-2001) and the DataSur database (2002-2015). Between 1998 and 2015, 95% (7,775 tons) of the antimicrobials were for VM use. Of these, 19% (1,480 tons) corresponded to quinolones, 35% (2,683 tons) to phenicols, and 46% (3,612 tons) to tetracyclines. For HM, of 381 tons imported, only 7% (28 tons) corresponded to phenicols, 11% (41 tons) tetracyclines, and 82% (312 tons) quinolones. Phenicols for VM increased from 3.2 tons between 2000-2003 to 1,606 tons between 2012-2015. This increase probably corresponds to use in aquaculture. For VM, 617 tons of quinolones were imported during 1998 to 2003, this decreased to 121 tons in 2010 to 2015. 77% (1,132 tons) of the quinolones imported for VM corresponded to aquaculture use. Between 2000-2015, 226 tons of tetracyclines were imported per year for VM in comparison with 2.5 tons average per year for HM. Our findings indicate that medically important antimicrobials are used indistinctly in HM as much as in VM. These observations, strongly suggest that in Chile, veterinary use of antimicrobial is the most important selective pressure for generating antimicrobial resistant bacteria.

Instituto de Farmacia, Facultad de Ciencias, Universidad Austral de Chile

CLP2-6

Activation of cellular response induced by immunobiotics protects salmonids against bacterial infections

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The explosive growth of salmon farming in the latest decades has brought huge gains to the country; however, the continuous outbreaks of infectious diseases have meant the implementation of vaccines, and antimicrobials to fight against pathogens. The use of probiotics capable of stimulating the immune response (immunobiotics), is a feasible tool to stimulate the immune system of mammals. However, their effects on salmonids are unknown. This strategy is non-invasive, does not require manipulation and use of a GRAS organism as a vehicle to deliver the immunostimulant. In the laboratory, a bacterium with probiotic properties was modified to activate the immune response in salmonids. To observe its effect, we fed three groups of salmonids, with different diets: G1 were treated with the improved probiotic, G2 were treated with the unmodified probiotic and, G3 does not receive any probiotic along with the experiment. Lysozyme activity in serum, quantification of anti and inflammatory cytokines expression in immune organs along with challenge assay with a bacterial pathogen were used to evaluate the effects of these treatments on the immune response. An increase in enzyme activity levels of lysozyme was observed from the fifth day after feeding on fish treated with the modified bacterium. It was also possible to observe an increase in the transcript levels of some cytokines such as IFN-γ, IL-12 and IL-6 in fishes treated with the modified bacteria. The increased expression of pleiotropic cytokines linked to the innate response, together with the level of serum lysozyme, and the protective effect in challenge assays suggest a stimulation of the cellular response, which allows reducing the bacterial load to levels under the threshold necessary to produce mortality. Altogether our result shows that immunobiotics are feasible tools against bacterial pathogens in Salmonids.

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CLP2-7

Model-driven engineering in *Pseudomonas putida* KT2440 for improved production of medium-chain-length polyhydroxyalkanoates on glycerol

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Background. Microorganisms produce Polyhydroxyalkanoates (PHAs) –a sort of biopolymer– under nutrient limited growth conditions, which accumulate as inclusion bodies in the cytoplasm of the cell as carbon and energy source. PHAs are considered a potential replacement of conventional plastics due to their similar mechanicals and physicals properties as the ones shown by thermoplastics. The soil bacterium *Pseudomonas putida* KT2440 can use glycerol, a by-product from the biodiesel industry, to synthesize this biopolymers amazing more than 30% of its cell dry weight as PHA. Driven by *in silico* simulation of the pathway topology of *P. putida* KT2440, we found several genetic targets (knockout) to increase the synthesis of mcl-PHA on glycerol, where by-product formation (malate and succinate) is avoided. In the present study, we will generate a knockout of the gene *ppc* encoding for a phosphoenol pyruvate carboxylase. Hypothesis: The knockout of the *ppc* gene increase PHA production in *P. putida* KT2440 growing in glycerol like the only carbon source. **Methods.** To test this hypothesis, we will generate a knockout mutant strain in *P. putida* KT2440 of the gene *ppc* through homologous recombination. For this purpose, we generated a construct by PCR Sewing, which was introduced into the pEMG plasmid by restriction sites. The pEMG-ppc fusion will be co-integrated into the genome of *P. putida KT2440* by a single cross over. Biopolymer content and secondary products obtained in the batch culture will be characterized using HPLC and gas chromatography–mass spectrometry. **Results.** We have successfully obtained the mutant and the total biomass is not affected under PHA production condition. Now, we will test the PHA production capacity of the mutant compared with *P. putida* KT2440 WT and further the characterization of the biopolymer obtained. **Conclusion.** The knowledge of how modulate the metabolism of *Pseudomonas* for one particular interest, allow us to obtain this specific mutant *P. putida KT2440Δppc* that through the redirection of carbon flux would be able to increase the PHA production.

CLP2-8

Genome analysis of species of the new bioleaching acidophilic bacterial genus *Acidiferrobacter*

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Biomining is the use of microorganisms for the industrial extraction of valuable metals and treatment of mine waste. *Acidiferrobacter* is a recently described genus of bioleaching bacteria. These are ca. 2 µm long and ca. 0.3 µm wide, non-motile slender rods, extremely acidophilic (optimal pH ~2) with some strains being slightly thermotolerant (optimal growth temperature ~38°C) and with moderate osmophilia. They are obligate autotrophs and facultative anaerobes that can grow by the oxidation of ferrous iron, sulfide, sulfur and metal sulfides such as pyrite. Currently there is one draft genome sequence available *Acidiferrobacter thiooxydans* strain ZJ (MDCF01). Our research group has sequenced two other strains, *Acidiferrobacter* SP-III/3 and *Acidiferrobacter thiooxydans* DSM 2392^T. Previous genome analysis and phylogenetic comparison of these strains has shown that strain SP-III/3 could be a new species different from strain DSM 2392^T. The objective of this work is to annotate and curate the genome sequences of the aforementioned strains with the purpose of identifying and reconstructing their metabolic pathways, with emphasis on those related to iron & sulfur oxidation, CO_2 and N_2 assimilation, extracellular polymeric substances (EPS) biosynthesis, and cell-to-cell communication mechanisms (i.e. Quorum Sensing, QS). Annotation of *Afb. thiooxydans* strains DSM 2392^T and ZJ; and *Acidiferrobacter* SP-III/3 was done using the server tool RAST and manually curated by using ClustalW, MAFFT as well as homology criteria with NCBI indexed databases. All strains share the iron oxidation *rus* operon and possess genes for sulfur and thiosulfate oxidation, including a *Dsr* and *Sox* operon. The strains also possess genes for assimilation of nitrate and N_2 fixation genes with *nif* and *fix* operons, having also genes for the assimilation of cyanide. CO_2 fixation shall occur through CBB/C2 carbon assimilation metabolism with a *cbbR* operon encoding for Rubisco and carboxysome proteins. Analysis also shows the presence of genes related to EPS (*pel* operon), capsule formation (related to group 1 and 4) and QS, which may be relevant for attachment to minerals, biofilm formation and cell-to-cell communication. This comprehensive study will provide new insights about *Acidiferrobacter* biology and its relevance in acidic environments where iron/sulfur/metal sulfide oxidation takes place.

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CLP2-9

Chloride ions induce oxidative stress in *Leptospirillum ferriphilum* DSM14647

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The chloride bioleaching is a method to recover copper from chalcopyrite ($CuFeS_2$), however, the chloride ion is known to be highly toxic for acidophilic bioleaching microorganisms. The toxicity of this ion is due to its capacity to cross the cell membrane therefore disrupting the inside positive membrane potential. Thus, the chloride ions produce an influx of protons and finally the acidification of the intracellular pH. Proteomic studies have shown that acidophilic microorganisms respond to chloride ions increasing the level of proteins of the electron transport chain, suggesting that the cellular respiration process might contribute to remove protons from the cytoplasm as a mechanism to restore the intracellular pH. An interesting effect of a higher respiratory rate could be an increasing in the production of the Reactive Oxygen Species (ROS), thus explaining the high toxicity of chloride ions to the acidophilic microorganisms. Hence, in this work we propose that chloride stress increase the ROS level and induce oxidative stress in the cell. To approach this hypothesis, we measured intracellular ROS level and activity of antioxidant proteins cytochrome c peroxidase (CcP) and thioredoxin (Trx) from *Leptospirillum ferriphilum* cultures exposed to NaCl. The effect of an antioxidant compound or a compatible solute was also evaluated. The results shown that *L. ferriphilum* challenged with NaCl 100 mM during 90 min increased ROS level in 230%. In agreement with these results, the CcP and Trx activity was increased four times. Furthermore, the pre-incubation with cobalamin (antioxidant compound) and the addition of hydroxyectoin (compatible solute) decreased significantly the ROS level in 76% and 61%, respectively, regarding with chloride stressed cultures. The pre-incubation with cobalamin increased both antioxidant activity in 25%. Nevertheless, the addition of hydroxyectoin restored these activities to similar level that control. Altogether, these results reveal a relationship between chloride stress and oxidative stress, involving the antioxidant response in the NaCl tolerance mechanism.

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CLP2-10

A transcriptomic approach about cytochromes and oxygen affinity in *Leptospirillum ferriphilum*

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Background. *Leptospirillum ferriphilum* is one of the most abundant ferrous iron oxidizer and ferric iron (leaching agent) producer in consortia inhabiting copper bioleaching systems. Electrons from iron oxidization are transported to cytochrome oxidases which use oxygen as final electron acceptor. It has been described different types of terminal oxidase with different oxygen-affinities. There is evidence that expression of high oxygen-affinity cytochromes are induced at low oxygen concentrations and vice versa. There are scarce evidence about cytochromes affinities with the O_2 in bioleaching species and it is an interesting research because of the high cost of forced aeration in bioleaching process. Two types of terminal oxidases (Cbb3 and bd-type) have been found in *L. ferriphilum* both described as high oxygen-affinity cytochromes. The goal of this work was to elucidate the role of these cytochromes in the adaptation of *L. ferriphilum* to variable aeration conditions. **Methods**. Transcriptomic of *cbb3* and *cydAB* genes, associated to cytochromes of *L. ferriphilum,* were studied in a bioleaching column inoculated with a MEL microbial consortium, in an industrial bioleaching heap (Minera Escondida-MEL) and in *L. ferriphilum* DSM14647 cultures. The input-airflow was intervened twice in the column test with output-O_2 concentrations ranging from zero to 15%. Industrial samples were obtained from strips with and without aeration in the heap. Culture tests were performed at three constant input-O_2 concentrations (3%, 10% and 23%). Relative quantification were performed by RT-qPCR using validated gene-specific primers, validated reference genes and Pfaffl model. Population dynamics and cell growth associated to O_2 availability was also analyzed. **Results**. The *cbb3* transcription was over-expressed only when O_2-flow was abruptly interrupted and no when cells were growing in constant low-O_2 levels. Lack of O_2 triggered a decrease in cell growth, microbial activity and *cydAB* transcription. **Conclusions**. An association between O_2 availability, population dynamics and transcriptional dynamics was observed. *cydAB* genes showed a transcriptional dynamic according with low O_2-affinity cytochrome different to the role reported in other species. Cbb3 cytochrome could perform different metabolic functions depending on the life history associated to O_2-availability of cells. The expression profile modeling will get knowledge for improving the bioleaching operation.

PROYECTO FONDEF IT13I20042

CLP2-11

Engineering *Halomonas elongata* for high-level production of PHB under unsterile conditions

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Background. Microbial polyesters or Polyhydroxyalkanoates (PHA), are a sustainable option to petroleum-based plastics since they can be obtained from renewable carbon substrates such as glucose and fatty acids. PHAs display various properties that can be modulated via various fermentation modes or genetic manipulation of the host strain. Microorganisms accumulate PHAs as energy/carbon reserves under nutrient imbalance e.g. high carbon concentration accompanied by the limitation of O_2, N, and P. However, one of the biggest obstacles to position PHAs in the market is their high cost of production at industrial scale. Therefore, the implementation of open system in the production process of PHA provides an alternative to reduce the costs associated with the energy required for sterilization. Within the wide array of microorganisms capable of producing PHA, *Halomonas elongata* is a gram-negative, halophile bacterium, which generates a particular type of PHA, known as poly (3-hydroxybutyrate) or PHB. It has been described that *H. elongata* tolerates up to 130 g/L of NaCl, opening a great opportunity for synthesizing PHB in unsterile systems. This high salt-tolerance is given by the synthesis of ectoine (protects the cell from osmotic damage). In turn, it has been proven that under culture conditions with high salt concentrations, a large part of the carbon flux is channeled to the production of ectoine, thus diminishing PHB accumulation. **Methods.** In this work we used *Halomonas elongata* DSM 2581 to produce PHB in unsterile culture system. For this, the bacterium was cultured at different NaCl concentrations 5, 30, 33, 50, 70, 80, 90, 100 and 130 (g/L) to assess important physiological parameters and PHB production. **Results.** We found the highest specific growth rate was 0.34 (1/h) when the cells grew on 70 (g/L) of NaCl. The PHB synthesis, at the different salt concentrations, was also assessed. In order to increase the PHA synthesis in *H. elongata*, we constructed a knockout mutant *H. elongata* strain *(ΔectA*), which tolerates up to 55 (g/L) of NaCl. **Conclusion.** The purpose of this study is the production of PHB in open culture systems, where the mutant strain shows promising PHB production performance in comparison to the wild-type *H. elongata* strain.

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CLP2-12

Withdrawn

CLP2-13

Effect of copper on the quality and quantity of the lipopolysaccharide from *Acidithiobacillus ferrooxidans* strains ATCC 23270 and ATCC 53993 grown in the presence of the metal

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Acidithiobacillus ferrooxidans is a chemolithoautotrophic and acidophilic bacterium, involved in the microbial consortium capable of bioleaching metals such as copper. This microorganism has the ability to live at very high metal concentrations and therefore, plays a pivotal role in bioleaching of minerals. One of the most abundant and important components of the outer membrane of Gram-negative bacteria such as *A. ferrooxidans* is the lipopolysaccharide (LPS). This molecule consists of an integral hydrophobic part known as lipid A, and a hydrophilic portion divided in the "core" and the O-polysaccharide or O-antigen. Being the most exposed molecule of the bacterium, LPS is in direct contact with the environment. Therefore, it can directly interact with metals present in the environment. Some reports indicate that modifications of LPS can affect the attachment of the bacteria to different surfaces and even their viability. We have previously found an increase in the level of some proteins related to LPS synthesis in *A. ferrooxidans* grown at high copper concentrations. To find out whether LPS modifies its quality and/or quantity under these conditions we used polyacrylamide gel electrophoresis and quantified transcriptional expression of the genes of interest by using qRT-PCR. This was studied in *A. ferrooxidans* strains ATCC 23270 and ATCC 53993. Preliminary results indicate that the quality of the LPS does not vary significantly when the bacteria are exposed to different concentrations of copper. On the other hand, the amount of LPS does vary depending on the metal concentration present in the medium.

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CLP2-14

Bioremediation of hydrocarbon polluted soils at semi-industrial scale

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Petroleum hydrocarbons are highly persistent in the environment and represent a significant risk for human health and biodiversity of ecosystems, affecting urban, industrial and natural areas. Frequently, hydrocarbon-contaminated sites remain polluted for long periods of time due to a lack of efficient bioremediation treatments. Those treatments are often based on bioaugmentation using exotic hydrocarbon-degrading microorganisms, or on biostimulation of the native microbial communities by adding nutrients to the soil. However, the main core of knowledge in such treatments mainly relies on lab scale studies, whereas the dynamic of degradation under more realistic conditions, such as in situ bioremediation, remains understudied. On the other hand, each polluted soil has its own physico-chemical particularities, making the design of the bioremediation approach a rather troublesome decision. In this sense, the research presented here aims to study the dynamic of bioremediation of hydrocarbon-polluted soils subjected to different treatments at a semi-industrial scale. The experiment was conceived as outdoor bioremediation systems, consisting in five biopiles, and carried out during 26 weeks. Each biopile was made of 400 L of contaminated soils subjected to four different treatments. Two biopiles were treated by bioaugmentation where a consortia of seven hydrocarbonoclastic bacterial strains was weekly added. Another two biopiles were treated by biostimulation adding organic amendments at the beginning of the process. Air was also supplied in the half of those treatments. The fifth biopile, without any intervention, was placed as control. Environmental and biological parameters such as humidity, pH and culturable heterotrophic bacteria and hydrocarbons were periodically monitored. During the experiment we observed a higher degradation rate along biostimulated treatments. As it was expected, the biopiles subjected to air-surplus presented a higher biodegradation rates than their counterparts, probably as a result of increase of oxygen that limits the oxidation process. Key factors determining the kinetic of degradation among the treatments are discussed. This study emphasizes the need for more detailed analysis in field studies, which are crucial to move on industrial bioremediation of soils polluted with hydrocarbons.

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CLP2-15

Lipids from a novel native *Thraustochytrium* strain: effect of temperature on the lipid composition

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Thraustochytrids are marine heterotrophic protist that have attracted the attention because of their ability to accumulate lipids with a high content of long chain polyunsaturated fatty acids (LC-PUFA), especially docosahexaenoic acid (DHA, C22:6n3). LC-PUFAs are distributed among structural lipids and lipid storage reserves. The exact role of LC-PUFAs in these microorganisms is not known; however it has been suggested that these molecules could promote fluidity to cell membrane. Cell membrane composition should be determined by the conditions (temperature, salinity, nutrient availability) of the natural environment where these microorganisms develop. The object of this work was evaluated the effect of growth temperature on the production and composition of lipids fractions in the biomass of a native *Thraustochytrium*. The microorganism was cultivated in shake flasks at three temperatures (5, 15 and 25°C). The growth medium contained yeast extract, sodium glutamate and maltose at 40 g/L; incubation was made for seven days at 150 rpm. The biomass growth and lipid composition were determined every 24 h. Biomass concentration was determined by gravimetric measurements; the residual concentration of maltose was determined by the phenol-sulfuric acid method. Lipids were extracted from the lyophilized biomass using the Bligh and Dyer method. Solid-phase extraction was used to separate the total lipids into neutral lipids, glycolipids and phospholipids. The fatty acid composition of the total lipids and lipid fractions was analyzed by gas chromatography. All the assays were carried out in triplicate. Experimental data was submitted to ANOVA to test the significance of the effects. As a function of temperature, biomass concentration (12.4±0.0 g/L) and the specific growth rate (0.021 h^{-1}) presented the highest values at 15°C. Total lipids increased during the exponential growth rate phase. The maximum lipid percentage in the biomass grown at 15°C was 21.1±1.6%. In this case, the total lipids were composed of 30.3±6.0% neutral lipids, 29.6±5.7% glycolipids, and 34.8±5.9% phospholipids. DHA (0.8 - 6%) and eicosapentaenoic acid (0.2 - 21%) were found in all lipid fractions. In conclusion their contents were significantly (p<0.05) affected by the temperature. PUFA profile of the phospholipids in the native thraustochytrid would suggest biotechnological and nutraceutical applications.

CLP2-16

Increment in growth rate and electric current generation under electrogenic and anaerobic conditions of *E. coli* mutants and involvement of NADH/NADPH by Flux Balance Analysis

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Background. In heterotrophic organisms, the central metabolic pathways allow the oxidation of the carbon source fulfilling two main functions: energy generation and biosynthesis. The reduced form of nicotinamide adenine dinucleotides (NADH and NADPH) are produced by specific dehydrogenases, and their consumption may affect the magnitude of the carbon flux of these reactions. Therefore the redox balance affect physiologic parameters such as growth rate and biomass yield. Moreover, the electrons originating from NADH can be transferred to external final acceptors, as in aerobic and anaerobic respiration. Thus, the reducing power generated by the metabolism, may be exploited through Microbial Fuel Cells (MFC), devices using an electrode as the final electron acceptor. In this way, MFCs converts the chemical energy from organic compounds into electric energy as a result of reactions catalyzed by bacteria. **Methods.** In this work we used *Escherichia coli* mutants bearing an NAD-dependent glucose-6-phosphate dehydrogenase and/or the deletion of glucose-6-phosphate isomerase, cultured under anaerobic and electrogenic conditions (with the use of Neutral Red). We determined physiological parameters at the exponential growth phase, which were used as constrains in Flux Balance Analysis (FBA), in order to study the involvement of the cofactors NADH and NADPH in the generation of electricity through MFC. **Results and Conclusions.** Compared to wild type, the *E. coli* mutants displayed incremented current under electrogenic and anaerobic conditions. Interestingly, in all the strains the growth rate is increased under electric current generation compared to anaerobic cultures in the presence of neutral red. The FBA analysis showed a redistribution of the fluxes at the central metabolic pathways. The involvement of NADH and NADPH in electricity generation, as well as the role of transhydrogenases are discussed.

Proyecto VID ENL012/16

CLP2-17

Synthesis of Quantum dots CdTe through Tellurite resistant bacterial strains

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Background. Cadmium Telluride (CdTe) Quantum Dots have several applications in different fields of industry because of their optic and electronic properties. Synthesis of these structures had been successfully performed by chemical methods. However, the procedures and mechanisms underlying microbiological synthesis of CdTe have not yet been clearly established. One of the main handicaps for these purposes is the elevated toxicity of the most soluble tellurium form, tellurite (TeO_3^{2}), whose MIC is as low as 0.004 M for *Escherichia coli*. Given the growing interest in nanostructure formation through biological processes is that tellurite resistant bacteria represent an interesting source for biosynthesizing CdTe Quantum Dots. There are several bacterial strategies for tellurite resistance and one of them is its reduction to the elemental form (Te^0) often through the formation of black deposits. In this line, tellurite-treated bacteria would need two additional electrons for CdTe formation (Te^{2-}); these could be provided in mineral oil-overlayed media, which would avoid volatile sulfide release and potentially increase the concentration of cellular thiol groups. **Methods**. Selected tellurite resistant strains were treated with potassium tellurite (K_2TeO_3) and cadmium chloride ($CdCl_2$) and their reduction abilities and CdTe formation were compared to those of the tellurite-sensitive bacterium *Escherichia coli* BW25113 under aerobic, mineral oil coated-cultures and anaerobic conditions. CdTe quantum dots were detected by fluorescence. **Results**. It was observed that tellurite resistant bacteria treated with potassium tellurite and cadmium chloride were able to reduce tellurite to its elemental form in the presence and absence of oxygen, exhibiting the typical blackening of cells because the formation of elemental tellurium. In mineral oil-overlayed culture media, the same strains reduced tellurite to telluride and formed CdTe, which were observed by the intrinsic fluorescence of quantum dots. *E. coli* did not form CdTe quantum dots in the tested conditions. **Conclusion**. In mineral oil-overlayed cultures, the greater reduction potential of tellurite resistant bacteria represents a good source for the biological synthesis of tellurium nanocomposites.

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CLP2-18

Characterization of the pangenome of *Acidithiobacillus ferrooxidans* uncovers differentiating aspects of intrinsic sublineages

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For many years, three validly described sulfur oxidizing *Acidithiobacillus* species – A. *thiooxidans*, *A. albertensis* and *A. caldus* - and one sulfur- and iron-oxidizer –*A. ferrooxidans* – were recognized to conform the genus. In recent years, three novel iron oxidizing species – *A. ferrivorans*, *A. ferridurans* and *A. ferriphilus* - have been acknowledged on the basis of a number of revised differentiating physiological characteristics and multi locus sequence analysis. Still, many isolates with varying phenotypic properties and cognate genetic traits remain to be adequately described. In the *A. ferrooxidans* linage, at least three sublineages (2A, 2B and 2C) have been recently described. While lineage 2A is represented by the type strain of the species and other well-characterized model strains, lineages 2B and 2C are less well studied. In this study, we use pangenomic strategies to provide a better understanding of the genome-level changes that drive ecological differentiation and lineage divergence within *A. ferrooxidans*-like strains (16 strains), while aiding in the elucidation of the taxonomic status of controversial strains. The two new sublineages identified (2B and 2C) on the basis of a few molecular markers, are clearly supported by whole genome sequence comparisons and genomic signature analyses. Each of the lineages has 8.5% (2A), 9.2% (2B) and 10.5 % (2C) of unique genes, many of which concur with mobile genetic elements signature genes. Metabolic reconstruction analyses indicate that differentiating traits between these variants include the flagellum, urea degradation/utilization systems, iron homeostasis related functions and restriction-modification systems. Inferences on the ecological significance of the uncovered features in linages adaptation and divergence are presented and discussed.

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CLP2-19

Poly-3-hydroxybutyrate (PHB) production from renewable carbon sources

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Polyhydroxyalkanoates (PHAs) are biopolymers synthesized by diverse bacteria genera as carbon and energy sources under stressful conditions. PHA granules are accumulated under unbalanced nutritional conditions. Poly-3-hydroxybutyrate (PHB) is the most studied PHA and has similar properties of polypropylene (PP), but its production has a higher cost. Low cost substrates as waste materials and by-products may be alternative carbon sources to reduce PHA production cost. The aim of this report, was to study the PHB production by *B. xenovorans* LB400 and *Janthinobacterium* sp. BmR6b using waste materials and by-products including glycerol, orange peels and beet molasses as carbon sources. Bacterial cells were grown in M9 medium with NH_4Cl (1 g L^{-1}) as nitrogen source, and waste glycerol, orange peels or beet molasses as carbon sources. Furthermore, used cooking oil was added as additional carbon source. Culture samples were collected after 24 and 48 h. Cells were freeze-dried and subjected to propanolysis. Propylesters obtained by propanolysis were analyzed by GC-MS. PHB was produced by *B. xenovorans* LB400 and *Janthinobacterium* sp. BmR6b from these waste materials and by-products. The addition of used cooking oil increased PHB production by strain BmR6b. Therefore, used cooking oil is an attractive substrate for the optimization of PHB production.

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CLP2-20

Performance of a low pH sulfidogenic bioreactor treating acidic mine water

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Mining contributes to water pollution through generation and uncontrolled release of acid mine drainage (AMD). This polluted water which runoff from operating and abandoned mines is characterized by low pH, elevated concentrations of transition metals and sulfate. One of the main limitations to use sulfate reducing bioreactors to treat AMD is that most species are highly sensitive to acidity, therefore it is necessary to avoid direct contact between acidic mine waters and the bacteria, which is normally achieved by using "off-line" systems. The additional engineering complexity required for "off-line" systems increases construction and operation costs. There have been few successful applications using acidophilic sulfate reducing bacteria populations, which can avoid this issue and allow direct metal precipitation as sulfide to occur within a single bioreactor unit. Here we describe the performance of a low pH sulfidogenic bioreactor inoculated with indigenous microbial communities for treating AMD. The inoculum was obtained from an anaerobic sediment found in an acidic river located in northern of Chile. The sulfidogenic system (2.3 L) was operated as a continuous flow mode unit for a period of over 100 days at 30°C and fed with synthetic water based on the chemical composition of the acidic river, characterized by extremely low pH (2.1) and zinc and iron as main transition metals (0.5 and 2 mM respectively). The feed liquor was supplemented with yeast extract (0.01%) and the concentration of glycerol (as electron donor) varied between 3 mM and 10 mM. The bioreactor pH was set at 4.5 initially and was increased, in stages to 6.0 during the experiment. The results show that zinc concentrations in liquors draining the bioreactor were below level of detection in most of the samples analyzed. By progressively increasing the concentration of glycerol added to the fed, it was possible to increase the removal of iron (70% of the total present), though more acetic acid (from 1 to 5 mM) was generated. Analysis of the microbial populations showed that they changed with varying operation parameters, and a known acetogenic sulfidogen (*Desulfoporosinus acididurans* strain USS-CC1) to become more dominant.

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CLP2-21

Microbiological study of the thermal water of the spa "Piscinas El Cachaco", Calacali, province of Pichincha Ecuador

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The thermal waters are ecosystems where they inhabit a variety of microorganisms that have adapted to the extreme conditions of temperature and concentration of salts. It has been postulated that the microbiota of each thermal Spa is characteristic. The objective was to determine the amount and types of microorganism present in the water of the "Piscinas El Cachaco" Spa, Province of Pichincha. Thermal water samples were collected in three areas of the Spa for a period of three months. Physicochemical tests were performed in each site using a multiparameter equipment. The samples were taken according to the NTE-INEN-2169 Ecuadorian rule. Microbiological analyzes were performed according to the APHA (2005) methodology and those recommended by Andueza (2007). The results show that in the well water that feeds the thermal pools, average values for coliform bacteria were 5 CFU / mL, aerobic mesophilic bacteria 3 CFU / mL and no *Staphylococcus* or mold and yeast cells were detected. In the thermal pools water mean values obtained for coliforms were 7 CFU / mL, mesophilic aerobic bacteria 2.58 X 10^2 CFU / mL, *Staphylococcus* 1.54 X 10^2 CFU / mL, yeast 5 CFU / mL, and no Molds were detected. For the natural spring waters, the mean values were coliform bacteria 1.06 X 10^2 CFU / mL, mesophilic aerobic bacteria 7.2 X 10 CFU / mL, *Staphylococcus* 8.9 X 10 CFU / mL, yeast 1.6 X 10 CFU / mL and molds 3 CFU / mL, 82% of the isolated bacteria were Gram negative, with the *Aeromonas* genus prevailing, while 18% were Gram positive, with the genus *Staphylococcus* prevailing. Three molds were identified as *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp. The results show little diverse microbiota in most areas of the Spa. Further studies are required to characterize the detected microbiota.

Proyecto: Biodiversidad microbiana en aguas termales de las provincias de Cotopaxis Chimborazo, Pichincha y Tungurahua. Financiado por el Instituto de Investigaciones y Postgrado. Universidad Central del Ecuador

CLP2-22

Global metagenomics of hotsprings microbial communities

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Metagenomics allows the assessment of the taxonomic and functional diversity of microbiomes. Hot springs are geothermally heated discrete habitat patches that cover large temperature gradients, which makes them a natural laboratory to test the effects of geographic barriers and temperature on patterns of diversity. Here, we analyzed a global database (five continents) of 23 hot springs metagenomes (Bacteria and Archaea) covering a wide gradient of temperature (32-98 °C) and pH (2.5-9.25). Our analysis identified ~1500 species, ~5000 genes and functions categorized in SEED, EggNOG and InterPro2GO databases, including a wide range of metabolisms. Results showed that species abundance and alfa diversity varied approximately unimodally with temperature and pH, while community similarity decreased with environmental dissimilarity. Whole gene and functional diversity did not varied with temperature and pH, except for less abundant ("rare") genes and functions. Species alfa diversity increased with area sampled and species beta (Sørensen) diversity decreased with geographic distance. Gene and functional diversity also increased with area sampled but their similarity did not decreased with geographic distance, except those "rare". We also reconstructed correlation networks of species and genes to characterize their associations based on common (overlapped) ecological niches. We found that the species network is characterized by having community structure evidenced by high modularity in contrast to the genes network, which showed low modularity. Altogether our results suggest that the species diversity of these microbiomes are highly determined by their evolutionary history, biogeography and environmental conditions, whereas only "rare" genes and functions varied with these factors. We suggest that temperature and pH determines abundance and diversity due to its role in biochemical kinetics, which ultimately also affects higher-level ecological processes. Otherwise, geographic limitation of species is due to hot springs are patched whereas the existence of lesser geographic limitation in genes is due to the existence of a core genome. Our results agree with patterns found in the human microbiome and other patched terrestrial ecosystems (e.g. salt marshes) but contrast with global patterns observed for the ocean, where there is lower geographic limitations.

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CLP2-23

Botrytis is associated with native and endemic plant in the Coquimbo Region

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Botrytis cinerea is a necrotrophic fungal plant pathogen that is the causative agent of grey mould in vineyards and can cause infections in a few hundred plant species and produce high impact in the local and world economy. However, there are few international studies that show the damage that this fungus can cause in native flora. The objective of this study was to characterize twenty six wild *Botrytis* strains isolated from native plants of Caleta de Hornos, Punta Teatinos, Quebrada de Talca, Peñón, Totoralillo and Fray Jorge National Park. The ITS region of rDNA was amplified using ITS1/ITS4 primers and then sequenced. ITS analysis of these isolates revealed that it corresponds to genus *Botrytis*. For further confirmation, nuclear protein-coding genes (G3PDH, HSP60, and RPB2) were sequenced. The resulting sequences showed 100% identity with those of *B. cinerea*. Complementary to this, *Botrytis* can also be clustered in two different groups, group I and group II, based on DNA polymorphism revealed by microsatellite markers, the *Botrytis* isolates were identified as member of group II. In addition, the wild *Botrytis* isolates were characterized by the presence of two mobile elements (TE's) named *Boty* and *Flipper*. The results indicate that the prevalence genotype was Transposa (with both active transposable elements). Infection assays were performed in apple fruits as well as leaves from *Phaseolus vulgaris* by inoculation with 5l droplets of conidial suspensions ($2.5x10^5$ conidia/ml Gamborg B5+2% glucose). The assays were made under standard virulence conditions including high humidity. The results shown that some isolates were more virulence in compare to reference strain. Antifungal resistance tests (Fludioxonil, Iprodione, Fenhexamid, Tebuconazole and Boscadil) show that 69% of the strains were sensitive to these compounds, however 31% of the strains were resistant to Fenhexamid or Boscadil. To our knowledge, this is the first study that report *B. cinerea* causing gray mold on native and endemic plant in the Coquimbo region and confirming that *Botrytis cinerea* is associated with them.

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CLP2-24

Role of the *Blautia* genus in the modulation of lactose tolerance as a member of the human gut microbiota

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Lactose intolerance is one of the most common gut disease conditions in worldwide populations (>70% of the world population). Variations in the presentation of this condition could be attributed to specific members of the human gut microbiota, as a key component of the interaction between the host diet and its health status. In this study, we leverage uBiome's gut microbiota dataset, which includes metadata information for all its users, to evaluate the effect of the microbiome composition in response to lactose tolerance. To identify candidate taxa that could be involved in this modulation, we compared a cohort of self-reported intolerant vs tolerant individuals, based on their gut microbiome compositions, obtained by sequencing the V4 region of the 16S rRNA gene. With this information and by using statistical testing after multiple hypothesis correction (FDR = 0.01), we identified that members of the genus *Blautia* have a higher abundance in lactose tolerant individuals, compared to lactose intolerant. This suggest that *Blautia* has the ability to thrive in this environment, and could play a role in the metabolism of lactose in the human gut, conferring them the ability to normally process dairy products. To explore this in more detail, we performed high-throughput sequencing of three *Blautia* isolates, on a MiSeq (Ilumina). Comparative genomic analysis was performed to identify differences in the functional potential of the strains. In addition, we used sequence similarity networks (SSN), to compare proteomes from sequenced organisms with all the obtained proteomes of *Blautia* species, to identify key members of lactose metabolism, such as glycoside hydrolases (GHA). In particular, we found a cluster with at least 15 novel GHA sequences, potentially associated with lactose metabolism. These results indicate that *Blautia genus* or at least certain species from this genus, could play a protective role in the modulation of lactose intolerance, conferring the ability to digest dairy products, to self-reported intolerant individuals. This ability could be a consequence of an increased number and diversity of GHA enzymes present in *Blautia* species.

CLP2-25

Construction and screening of metagenomic libraries derived from rhizosphere of two Antarctic plants for identification of genes conferring tolerance to lignocellulose-derived inhibitors

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The bacterial communities of Antarctic soils have been of great scientist interest in the last decades since extreme environments harbor microorganisms with unique adaptations and metabolisms. The understanding of how they cope with the harsh environmental conditions is an important step for the discovering of novel molecular mechanisms which otherwise, will remained unknown in the most abundant uncultured microbial fraction. In turn, lignocellulosic biomass has been investigated as a renewable non-food source for production of biofuels. A significant technical challenge to using lignocellulose is the presence of microbial growth inhibitors generated during pretreatment processes which are collectively known as lignocellulose-derived inhibitors (LIDs). In this work, we have explored by functional metagenomic the genetic reservoir for LIDs-resistance determinants which extant in the rhizosphere of the unique two vascular plants (*Colobanthus quitensis* and *Deschampsia antarctica*) growing on coastal soil during Antarctic austral summer. The metagenomic DNA from each rhizosphere was extracted and two small-insert libraries were constructed using the high-copy number pBluescript SKII+ vector and *E. coli* strain DH10B as host. Approximately $1.1x10^6$ recombinant clones were obtained within these libraries. Furthermore, a total estimation average of 2.4 Gb of environmental DNA was cloned per library which is considering a representative value for this type of environment, since an average of 4 Mb per genome is estimated. The libraries were subsequently amplified and the plasmids of 10 random clones were used to estimate an average insert size of 4.5 kb of the cloned environmental DNA (ranging between 1.0 and 7.0 Kb). Using a combined strategy through cloning and heterologous expression of the environmental DNA, we were able to demonstrate the phenotypic detection of several clones showing an increased tolerance to five different LDIs: 5-hydroxymethylfurfural, furfural, 4-hydroxybenzaldehyde, vanillin and syringaldehyde. Sequencing and ORF sub-cloning is currently undergone in order to identify clearly which heterologous gene(s) could be involved in LIDs-resistance. This work shows the utility of DNA libraries constructed from Antarctic environments to uncover novel genes with potential to be exploited in deployment of complex prokaryotic phenotypes as those required for efficient utilization of lignocellulose biomass.

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CLP2-26

Fermented soybean meal increases lactic acid bacteria in gut microbiota of Atlantic salmon (*Salmo salar*)

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The main goal of the present study was to address the effect of feeding fermented soybean meal-based diet to Atlantic salmon on gut microbiota. Further, expression of genes of interest, including cathelicidin antimicrobial peptide (*cath*), mucin 2 (*muc2*), aquaporin (*aqp8ab*) and proliferating cell nuclear antigen (*pcna*), in proximal intestine of fish fed either experimental diet was analyzed. Three experimental diets, including a control fishmeal (30% FM), soybean meal (30% SBM) or fermented soybean meal diet (30% FSBM) were randomly assigned to triplicate tanks during a 50-day trial. The PCR-TTGE showed microbiota composition was influenced by experimental diets. Bands corresponding to genus *Lactobacillus* and *Pediococcus* were characteristic in fish fed the FSBM-based diet. On the other hand, bands corresponding to *Isoptericola, Cellulomonas* and *Clostridium sensu stricto* were only observed in fish FM-based diet, while *Acinetobacter* and *Altererythrobacter* were detected in fish fed SBM-based diet. The expression of *muc2* and *aqp8ab* were significantly greater in fish fed the FSBM-based diet compared with the control group. Our results suggest feeding FSBM to Atlantic salmon may 1) boost health and growth physiology in fish by promoting intestinal lactic acid bacteria growth, having a prebiotic-like effect, 2) promote proximal intestine health by increasing mucin production and 3) boost intestinal trans-cellular uptake of water. Further research to better understand the effects of bioactive compounds derived from the fermentation process of plant feedstuff on gut microbiota, and the effects on health and growth in fish is required.

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CLP2-27

CRISPR-Cas systems reveal host-pathogen interaction in the thermal environment of Porcelana (Chilean Patagonia)

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The thermal system Porcelana, located in northern Chilean Patagonia, has a temperature gradient ranging from 48°C to 66°C. This extreme environment for life is dominated by communities of photoautotrophic and diazotrophic microbes that form a coherent mat with high biomass. Metagenomic studies have shown the coexistence of different thermophilic bacterial species and viruses. Porcelana microbial community is an excellent model of ecological studies because its low diversity compared to other environments. However, it is complex to infer the relevance of the host-pathogen interaction that could exist between bacteria and viruses due to the absence of viral isolates. In spite of this, one approach is the study of CRISPR-Cas systems, which correspond to a mechanism of bacterial adaptive immunity, where different viral genomic fractions can be actively incorporated into the bacterial cell as memory for future encounters. These molecular systems have been observed in genomes of Porcelana isolates. Here we present the analysis of the diversity of CRISPR-Cas systems in the thermal gradient (48-66°C) of Porcelana using a metagenomic approach. Also we dissect their relationship with metaviromes obtained for the same place. We search for all Cas proteins described to date and for the viral sequences that are observed stored in bacterial genomes as memory immunity system (spacers). Finally the viral elements that are attacked by CRISPR-Cas systems were analyzed. A large diversity of CRISPR-Cas systems represented by a large number of CRISPR arrays and different types of Cas proteins (some of unknown function) were found, with this systems targeting against different viral factors. The viral targets vary according to the temperature in the gradient and are mostly unknown. These results demonstrate the relationship of the CRISPR-Cas systems with the metaviroma, revealing the presence of a host-pathogen interaction in this thermal system. Furthermore, the presence of Cas proteins with unknown function, as well as non-annotated viral factors, could suggest the existence of an endemism characteristic of this extreme environment.

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CLP2-28

Signature of the nose microbiome across geographical and temporal changes

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Since the Human Microbiome Project (HMP) characterized the human microbiota of healthy subjects, multiple efforts have been made in order to elucidate how the microbiome impacts in human health. In this context, uBiome is a leading microbial genomics company that in collaboration with tens of thousands of citizen scientists,has built the largest human microbiome database. Nasal passages were one of the major body sites considered in HMP. This sample type has the characteristic that is in permanent contact with the environment, and which microbiome has been presumed of having promising roles in aspects of many diseases, such as rhinosinusitis or allergies. Although the nasal microbiome has potential importance in human health, the variations of taxa abundance considering different large sets of samples or environmental conditions remain unexplored. In this work, we look the diversity of 3,503 nasal microbiome samples, with the goal of exploring associations between the nasal microbiome and environmental factors. Samples were self-collected by individuals whose gave informed consent. We subsetted the subjects considering seasons and climates, according to sampling dates. DNA was extracted using the ZymoBead genomic DNA kit and the V4 region of the 16S rRNA gene was amplified with barcoded primers. Paired-end sequencing of the samples was performed on the Illumina™ NextSeq500 platform. Downstream bioinformatics analysis was performed after chimeras removal by aligning both ends to a SILVA v123 amplicon dataset that was previously manually curated. Identification of specific target, was carried out using metrics previously developed in our company. Taxonomic counts and abundance were analyzed in combination with the sample metadata. This can include, geographical location, sampling time, and other parameters obtained from the answer of the user to the survey questions. It is has been described that most of the species in the nasal passages are from the genus *Staphylococcus*, however our results revealed that there are also other taxas in common between seasons, such as *Corynebacterium* and *Propionibacterium.* We believe these results will enhance the understanding of the role of nasal microbiome in human health.

CLP2-29

Participation of *Salmonella* Typhimurium sensors kinase in response to oxidative stress

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The two-component system (TCS) are mechanisms that are conserved between species ranging from archaea to bacteria. These systems are composed by a sensor kinase (SK) generally attached to the outer membrane and a response regulator (RR) which has DNA binding domains that regulates the gene expression. The SK are activated by environmental signals, such as acidity, osmolarity, nutrients, reactive oxygen species (ROS), among others. This causes the SK autophosphorylation and the transphosphorylation to the RR. In our laboratory, we work with TCS ArcAB of *Salmonella* Typhimurium (*S.* Typhimurium) and we showed that this system is required for the survival and response to oxidative stress, regulating different genes involved in metabolism, virulence, detoxification, etc. However, the genetic regulation under oxidative stress are independent of each members of the system. In addition, previous studies have demonstrated cross-talk between different TCS under different conditions. We hypothesize that the RR ArcA of *S.* Typhimurium works with a noncognate SK in oxidative stress conditions. In this way, we evaluated the behavior of mutant in all SK of *S.* Typhimurium through the sensibility, growth behavior, survival and ROS accumulation against toxics compounds generated by hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl). The analysis showed eight candidates (Δ*barA*, Δ*creC*, Δ*ttrS*, Δ*cpxA*, Δ*dpiB*, Δ*srrB*, Δ*zraS* and Δ*kdpD*) that have a similar behavior that Δ*arcA* and Δ*arcB* strains under oxidative stress conditions described. Furthermore, we evaluated the resistance to the oxidative burst in murine macrophages through the determination of adherence and invasion of these candidates, finding a high sensitivity of the SK mutants strains inside phagocytic cells. Finally, we measured the gene expression of these candidates under oxidative stress produced by H_2O_2 and NaOCl in the wild type, Δ*arcA* and Δ*arcB* strains, indicating that the SK genes change their expression under these conditions. Our results showed the potential candidates that respond to ROS stress in a similar way as the TCS ArcAB, suggesting a new role in the adaptation and survival in the oxidative stress of *S.* Typhimurium. Also, our results suggest a putative role of the SK candidates in the infective process of *Salmonella* on phagocytic cells.

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CLP2-30

Identification of the *zot* gene in highly cytotoxic *Vibrio parahaemolyticus* strains of clinical and environmental origin

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Vibrio parahaemolyticus pathogenic strains have a large arsenal of virulence factors being the toxins TDH and TRH and the Type III Secretion System-2 (T3SS2b/a) the most important. However, diarrhea cases associated with strains lacking these classical virulence factors have been reported in many countries suggesting that they are able to produce disease by alternative mechanisms. The objective of this work was to identify, through bioinformatic analysis, the genetic determinants that could be related to the virulence of these strains. The genome of four *V. parahaemolyticus* tdh/trh/T3SS2b/a negative strains and highly cytotoxic in cell culture of Caco-2 (clinical PMC53.7 and three environmental PMA1 .15, PMA2.15 and PMA3.15 strains), were sequenced by NGS. Also, three non-cytotoxic strains of environmental origin (PMA11.14, PMA12.14 and PMA31.14) were used as controls. The analysis of the accessory genome of all strains showed that only highly cytotoxic strains PMC53.7, PMA1.15 and PMA3.15 harbored prophage-related elements encoding a gene related to zonula occludens toxin (zot). In *Vibrio cholerae*, Zot increases the intestinal epithelial permeability by interaction with the cellular receptor zonulin. Its action is mediated by a cascade of intracellular events leading to the disassembly of the intercellular tight junctions. In an attempt to determine the contribution of Zot to the virulence of *V. parahaemolyticus* strain PMC53.7 we evaluate its expression during infection assays in Caco-2 cell culture. The analysis of zot expression by qPCR showed detectable levels of expression after 2 hours post-infection which increased significantly at 4 hours. Interestingly, the *zot* expression was correlated with the cytotoxicity results obtained during the infection. The results suggest that Zot of *V. parahaemolyticus* could play a role in the virulence of this bacteria.

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CLP2-31

Identification of a new open reading frame from *DbPAD* gene, involved in the production of 4-vinylphenol on the wine-spoilage yeast *Brettanomyces bruxellensis* LAMAP2480

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The process of wine elaboration includes the action of several yeasts, being *Saccharomyces cerevisiae* that provides the main sensorial characteristics for this product. However, some yeasts alter drastically the quality of the final product. Yeasts belonging to the genus *Brettanomyces* are considered the main contaminating microorganism in the wine industry, due to their ability to produce volatile phenols from the hydroxycinnamic acids (*p*-coumaric acid, ferulic acid and caffeic acid) which are naturally found in the vinification process. The production of volatile phenols by *Brettanomyces* spp. involves the sequential action of two enzymes. First, a phenylacrylic acid decarboxylase (PAD), which converts the hydroxycinnamic acids into vinylderivates and posteriorly a vinylphenol reductase (VR) which reduce to ethylene derivatives. Previous studies performed by our laboratory has reported the sequence of the gene *PAD1* in *Brettanomyces bruxellensis* (*DbPAD*), which encodes a phenylacrylic acid decarboxylase that transforms *p*-coumaric acid into 4-vinylphenol. Bioinformatics studies indicate the presence of a second open reading frame (ORF) called *DbPAD2*, belonging to the *DbPAD* gene, which would code for an enzyme with the same activity. With this background, the objective of this work was to evaluate the ability of *DbPAD2* to generate a functional PAD. For that, we cloned the *DbPAD2* ORF into the vector YEpACT4 and its was subsequent expressed in *S. cerevisiae* under the control of *B. bruxellensis ACT1* promoter. The increase in 4-vinylphenol production was demonstrated in comparison to the control strain, which was indicated of the functionality of PAD encoded in *DbPAD2*. Along with this, by the *in silico* translation of *DbPAD2*, it was determined that the protein obtained has an amino acid sequence possessing the catalytic site of PAD, but with a smaller overall size due to the loss of 45 residues at the amino- terminal. Finally, the three-dimensional modeling of the protein encoded by *DbPAD2* showed similar folding to PAD, which would allow to suppose that *B. bruxellensis* LAMAP2480 produces two isoforms of the enzyme. Thus, this study allows to establish the biological bases involved the mechanism by which *B. bruxellensis* is able to modulate its gene expression to produce the aromatic defect in wines.

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CLP2-32

Functional characterization of c-di-GMP pathway in *Acidithiobacillus* species by comparative proteomic of silvestre and null-mutant strains

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Metagenomic and metatranscriptomic reports from acid mining drainage and terrestrial hot springs strongly emphasized the taxa of *Acidithiobacillus* as a predominant structural and active player in acidophile communities ranging from 6ºC and 90ºC. In addition it is well established that bacterial attachment on minerals increases leaching activities due to the formation of a "reaction space" between the metal sulfide surface and the cell. Then our laboratory is focused on deciphering the molecular mechanisms involved in the regulation of biofilm formation in acidophilic leaching bacteria. Our group has recently reported that c-di-GMP a central secondary metabolite for promoting biofilm formation pathway is widespread in *Acidithiobacillus* suggesting a common and ancestral role into the class *Acidithiobacillia*. We was also successful to develop null-mutant strains in both *At. caldus* and *At. thiooxidans* c-di-GMP pathway. Then to identify molecular network regulated by c-di-GMP pathway in *Acidithiobacillus* species we are currently performing proteomic analysis to compare wild type and null-mutant strains proteomic profile. Here we will report preliminary results and first hypothesis regarding c-di-GMP pathway and molecular network involved in biofilm formation in acidophile.

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CLP2-33

The Toxin-Antitoxin system YafQ-DinJ from *S.* Typhimurium participates in intracellular survival.

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Background. *Salmonella* Typhimurium is a facultative intracellular pathogen that can produce from a self-limiting gastroenteritis to a complex systemic disease. *S.* Typhimurium harbors genes that encode Toxin-Antitoxin (TA) systems, which mainly participate in stress response. In presence of stress, the relative toxin accumulation increases compared with the antitoxin, negatively affecting the metabolism and the bacterial replication to resist the adverse conditions through the formation of persister cells. Nevertheless, it has been described that some TA systems also participates in motility, biofilm formation, and in virulence through the formation these persister cells in epithelial cells, as described for *E. coli* that the YafQ-DinJ system. Although the YafQ-DinJ system is also present in *S.* Typhimurium, its role in motility, biofilm formation, and virulence has not been explored. **Methods**. We constructed the pBAD::*yafQ* plasmid by cloning *S.* Typhimurium *yafQ* (encoding an endoribonuclease toxin that prevent bacterial replication) into pBAD under an arabinose-inducible promoter to determine whether the expression of this toxin exerts an effect in the bacterial growth. Furthermore, we constructed the *S.* Typhimurium 14028s Δ*yafQ-dinJ* mutant using the allelic exchange method, and we studied motility, biofilm formation and invasion and proliferation in epithelial cell lines (HEp-2, HT-29). **Result**. The artificial expression of *yafQ* negatively affected the growth of *Salmonella* Typhimurium, both in liquid and solid media, suggesting that the YafQ-DinJ system is functional in this serovar. In addition, we found that the *S.* Typhimurium 14028s Δ*yafQ-dinJ* mutant exhibited a decreased motility and an increased biofilm formation compared with the otherwise isogenic *S.* Typhimurium WT. Finally, we found that the lack of the *yafQ-dinJ* genes, negatively affect both the invasion and proliferation in HEp-2 and HT-29 epithelial cell lines. **Conclusion**. Our results show that YafQ-DinJ participate in motility, biofilm formation, and invasion and proliferation in epithelial cell lines, suggesting a role in virulence.

FONDECYT Regular 1171397

CLP2-34

Natural diversity in the activation of the TORC1 signaling pathway by glutamine in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae is the main species responsible for the alcoholic fermentation in the transformation of grape must into wine, being one of the main problems the deficiency of nitrogen sources in the must, which can lead to stuck or sluggish fermentations. A major challenge is to identify the genetic basis underlying the phenotypic variability in nitrogen consumption and metabolism, with emphasis on the study of the TORC1 signaling pathway, given its central role in responding to nitrogen availability and influencing growth and cell metabolism. However, no clear mechanism has been identified by which the pathway is activated in the presence of nitrogen sources, with the study of allelic diversity appearing as an alternative to identify genes involved in this process. Although there are methods to evaluate the activation of the TORC1 pathway in the presence of nitrogen sources, these are time-consuming, making difficult to analyze large numbers of strains. Therefore, a new microculture method was developed using the luciferase gene as a reporter, which was tested in strains representative of clean lineages described in *S. cerevisiae* (North American ´NA´, Sake ´SA´, West African ´WA´ and Wine/European ´WE´). For these four strains, the activation of the TORC1 pathway by a proline-to-glutamine upshift was evaluated by traditional methods based on Western blot (Sch9 and Rps6 phosphorylation), verifying that there are phenotypic differences between the different representative strains. These results were then compared with the obtained with the new method developed, being concordant between them, showing the same phenotypic diversity among strains. In particular, the SA and WE strains showed extreme phenotypes, with the SA strain having the higher TORC1 activation, whereas the NA and WA strains showed similar intermediate phenotypes. These results open the possibility of using the pair WExSA as parental strains of a recombinant population, in order to detect the molecular bases that determine the activation differences of the TORC1 pathway in *S. cerevisiae* through a QTL mapping.

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CLP2-35

An altered brain inflammatory status causes behavioral alterations after infection by the human respiratory syncytial virus

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Background. The human Respiratory Syncytial Virus (hRSV) is the most common infectious agent affecting children younger than two years old, causing a significant increase in hospitalizations during the winter season due to bronchiolitis and pneumonia. Despite the devastating effects of this virus in infants, an efficient vaccine and treatments against this pathogen are not yet commercially available. Previously, we showed that hRSV was detected in the lungs as well as in the brains of mice exposed to the virus. Here, we show that hRSV infection promotes monocyte infiltration and induces the expression of pro-inflammatory cytokines in the central nervous system (CNS) of infected mice. **Methods**. Mice were intranasally infected with $1x10^6$ PFU of hRSV or mock, we evaluated the infiltration of inflammatory cells into CNS was evaluated 3 days after hRSV challenge. Moreover, we evaluated the pattern of cytokine expression by RT-qPCR at different days post-infection. Additionally, we performed Marble Burying tests at 60 and 90 days post-infection and also the cytokine expression was analyzed by RT-PCR. Further, we evaluated the ability of hRSV to infect CNS cells both *in vivo* and *in vitro*. We performed immunofluorescence of brain tissue of hRSV infected mice. Also, we evaluated the infection in human and murine astrocyte primary cultures. **Results**. A significant down regulation of CD200 and IFNα was seen at day 3 post-infection. Also, we found an elevated expression of pro-inflammatory cytokines such as IL-6 at different days after hRSV infection. Further, we evaluated the ability of hRSV to infect CNS cells both *in vivo* and *in vitro*. We performed immunofluorescence of brain tissue of hRSV infected mice and found that astrocytes were infected by the virus. Also, we evaluated the infection in human and murine astrocyte primary cultures and observed the peak of viral load at 24 h post-infection. **Conclusion.** Our data support the notion that this pathogen, besides causing major respiratory symptoms in the host, can impair the proper CNS function due to local inflammation.

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CLP2-36

FNR and ArcA regulate lipid A hydroxylation in *Salmonella* Enteritidis by controlling *lpxO* expression in response to oxygen availability

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Lipid A is the hydrophobic anchor of lipopolysaccharide in the outer membrane of Gram-negative bacteria and corresponds to the bioactive moiety of this complex molecule. Lipid A has a dynamic structure that can undergo covalent modifications in response to changes in environmental conditions. For instance, LpxO catalyzes the hydroxylation of lipid A in *Salmonella* in a reaction that requires oxygen as substrate. This process should be tightly regulated, since an appropriate level of lipid A hydroxylation is crucial for *Salmonella* virulence. However, the regulation of *lpxO* expression has not been fully addressed. Adaptation to changes in oxygen availability is an important challenge that *Salmonella* faces during infection, and transcriptional factors FNR and ArcA are the main regulators of this process. The aim of this study was to determine if *lpxO* expression and lipid A hydroxylation in *S.* Enteritidis are regulated by oxygen availability, and the role played by FNR and ArcA in these processes. As revealed by mass spectrometry, hydroxylated lipid A species in the LPS were more abundant than non-hydroxylated counterparts when wild-type *S.* Enteritidis was grown aerobically. On the contrary, non-hydroxylated lipid A species were the most abundant when wild-type bacteria was grown anaerobically. In addition, by qRT-PCR and *lacZ* transcriptional fusion analyses we confirmed that *lpxO* expression is higher when wild-type bacteria are grown aerobically than anaerobically. In contrast, the observed oxygen-dependent lipid A hydroxylation was lost in a Δ*fnr* mutant and in a strain that overexpresses *arcA* (WT/parcA), suggesting a regulatory role for FNR and ArcA in this process. Furthermore, the differential expression pattern of *lpxO* in aerobiosis versus anaerobiosis was severely affected in WT/parcA, Δ*fnr* and Δ*arcA* mutant strains. Finally, electrophoretic mobility shift assay revealed that FNR and ArcA directly bind to *lpxO* promoter. In conclusion, our results strongly suggest that FNR and ArcA regulate lipid A hydroxylation in *S.* Enteritidis by controlling the expression of *lpxO* in response to oxygen availabity.

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CLP2-37

Comparative analysis of different routine method for quantification of *Piscirickettsia salmonis*

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Background. *Piscirickettsia salmonis* is the aetiological agent of Salmonid Rickettsial Syndrome (SRS) in salmonids. This pathogen is an intracellular facultative Gram-negative bacterium that infects macrophages and hepatocytes of salmonids. Actually, *P. salmonis* cause the most prevalent bacterial disease in Chilean salmon Aquaculture. *P. salmonis* was first isolated and identified in several salmonid cells line and recently was reported its growth in liquid or solid free-cell artificial medias. However, the quantification methods used by research groups show difference in sensibility and not all techniques have the ability to discriminate the artifacts resulting in variable results. In this work, we compared several methods to quantify bacterium and we evaluated the ability to discriminate artifacts as cellular-debris. **Methods**. The cytopathic effect (CPE) produced in SHK-1 and CHSE-214 cell lines infected with *P. salmonis* strain was evaluated after 8, 12, 15 and 19 days post-infection by optical microscopy. At the same times, *P. salmonis* was quantify at infection supernatants by qPCR directed against 16S rDNA gene, *BacLigh* flow-cytometry kit, count in Petroff-Hausser chamber and serial dilutions of colony forming units (CFU) in CHAB agar. The reproducibility and sensibility of different methods was performed by linear regression of serial dilution of samples inter and intra experiments. **Results**. The CPE observed both SHK-1 and CHSE-214 cell lines infected by *P. salmonis* has a correlation with the quantity of bacterium presents at supernatant in all quantification methods analyzed. Although all quantification methods have a good detection sensibility, the quantification by qPCR and by count in Petroff-Hausser chamber over-estimated in two and one magnitude order respectively the number of bacterium present in infection supernatants compared to serial dilutions of CFU in CHAB agar-plates. Different is the case of quantification using *BacLigh* that have similar results to quantification by serial dilutions of CFU in CHAB agar-plates but with the advantage that results was obtained in 2 hour versus 7-10 days respectively. **Conclusion**. The quantification by *BacLigh kit* was the best method evaluated to quantify *P. salmonis* present in cell cultures with advantages as the simplicity in use, good sensibility, reproducibility, fast and ability to discriminate live and dead bacteria.

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CLP2-38

Heat shock protein 60 (Hsp60) as a putative effector translocated by secretory pathways of *Piscirickettsia salmonis*

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Piscirickettsia salmonis is the causative bacterial agent of piscirickettsiosis, a systemic fish disease that significantly impacts the Chilean salmon industry. This bacterium possesses a type IV secretion system (T4SS), several proteins of T3SS, and a single heat shock protein 60 (Hsp60). It has been suggested that due to its high antigenicity, the *P. salmonis* Hsp60 could be surface-exposed, translocate across the membrane, and (or) secrete into the extracellular matrix. This study tested the hypothesis that *P. salmonis* Hsp60 is a secreted putative virulence effector protein. Several bioinformatics approaches were used to assess Hsp60 translocation by the T4SS and T3SS systems. Immunogold electron microscopy and proteomic analyses suggested that although *P. salmonis* Hsp60 was predominantly associated with the bacterial cell cytoplasm, Hsp60-positive spots exist on the bacterial cell envelope. IgY antibodies against *P. salmonis* Hsp60 protected SHK-1 cells against infection. These data support the hypothesis that small amounts of Hsp60 must reach the bacterial cell surface in a T3SS- and T4SS-independent manner, and remain biologically active during *P. salmonis* infection likely mediating adherence and (or) invasion.

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CLP2-39

Evaluation of the functionality of lysosomes of Atlantic salmon macrophages infected with *Piscirickettsia salmonis*

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Background. *Piscirickettsia salmonis* is a facultative intracellular bacterium that has been suggested infects macrophages and evades immune response avoiding its lysosomal degradation. Unfortunately, there is a lack of scientific information on the mechanisms used by this bacterium to overcome infected–cell responses and survive to induce a productive infection in macrophages. For this reason, our work was focused in the evaluation of functionality of lysosomes of Atlantic salmon macrophages infected with *P. salmonis*. **Methods.** The pH of lysosomes of infected macrophages was analyzed using the Lysosensor Yellow/Blue™ probe that allow us differentiate an acid pH from a neutral by confocal microscopy. The proteolytic activity of lysosomes of infected macrophages was determined using DQ™-BSA-Green probe that are strongly self-quenched. Upon proteolysis digestion results in dequenching, releasing fluorescence detected by confocal microscopy. Complementary, we analyzed transcripts expression of lysosomal non-oxidative response markers, such as cathepsin D, B-glucuronidase, acid pPhosphatase and the antimicrobial peptide Hepcidine by RT-qPCR. Additionally, we analyzed the mRNA expression of effector proteins, DotB and IcmK, associated to Dot/Icm type IV-B secretion system from *P. salmonis* by RT-qPCR. **Results.** In macrophages infected with *P. salmonis* we observed that pH was not acidic remaining in a neutral range similar as the obtained in non-infected macrophages. By the contrary, in macrophages incubated by 24h with *P. salmonis* fixed with PFA we observed a pH-acidic range in lysosomes. Similar results were obtained when we analyzed the proteolytic activity in macrophages infected with *P. salmonis*, in which we observed a lower fluorescence intensity associated with BSA degradation in macrophages respect to proteolytic focus observed in macrophages incubated with *P. salmonis* fixed with PFA. Expression transcripts of four non-oxidative responses markers was increased either macrophages infected by *P. salmonis* and incubated with bacterium fixed with PFA compared to non-infected macrophages. Interestingly, the transcripts expression of *dotB* and *icmK* was increased when *P. salmonis* infects macrophages. **Conclusion.** Our results suggest that *P. salmonis* modulates the lysosome activation, possibly by a mechanism mediated by Dot/Icm type IV-B secretion system used by *P. salmonis* to promote intracellular bacterial survival.

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CLP2-40

Platinum-based antineoplastic drugs promote SG assembly and impact negatively on HIV-1 gene expression

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Under different types of stress, the cell activates several defense mechanisms to survive, or initiate apoptosis. For example, cells initiate processes leading to both the shut-off of mRNA translation and the assembly of Ribonucleoprotein Complexes (RNPs) called stress granules (SGs). It has been evidenced that its formation plays a role in the modulation of the viral replication, nevertheless, many viruses have developed mechanisms to avoid their formation during the infection. Human Immunodeficiency Virus type 1 (HIV-1) is capable to blocks the formation of SGs favoring the viral replication. However, under conditions in which HIV-1 is not able to suppress the formation of SGs, it showed negative consequences on viral production and its infectivity. The Platinum-based antineoplastic drugs have the ability to interfere with DNA repair mechanisms, causing DNA damage and subsequently, stimulating the SG assembly. We evaluated the effect of Platinum-based drugs (Cisplatin and Carboplatin) on HIV-1 expressing U2OS cells that constitutively express stress granules marker, G3BP1-GFP. Both drugs generated the assembly of G3BP-aggregations in HIV-1 expressing cells, decreasing the expression of mayor viral structural protein Gag. These results showed that anti-neoplastic agents could have a potential effect on viral gene expression.

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CLP2-41

Analysis of virulence factors of Chilean *Pseudomonas syringae* pv. *actinidiae* isolates: causal agent of bacterial canker of Kiwifruit

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Background. *Pseudomonas syringae pv. actinidiae* (Psa) is a phytopathogen that causes a severe economic losses in kiwifruit production of worldwide, including Chile. Phylogenetic analysis by MLST and Rep-PCR suggest Chilean Psa isolates are genetically homogenous. In this work, 18 Chilean Psa isolates obtained from VII and VIII regions, were characterized according to virulence factors such as motility, biofilm formation, indole production, and presence of effectors genes. **Methods.** Swimming and swarming motility were evaluated using LB medium with different agar compositions at 72 h post-inoculation. Biofilm production in surface adhered were determined by microtiter dish assay after 7 days of incubation, while air-liquid interface in static conditions were observed at 96 h post- inoculation. Tryptophan-dependent indole production was evaluated using the Salkowski assay. Presence indole production-pathway and effectors genes were analyzed by PCR reactions with specific primers. **Results.** All Chilean Psa isolates had swimming motility and only for Psa 889 isolate the movement were reduced. Swarming phenotype were reduced for Chilean Psa isolates and only Psa 510 had a slightly greater displacement. Psa isolates had a lower ability to produce a surface-biofilm, however, a thin layer of cell aggregates air-liquid interface were observed. Indole production by tryptophan-dependent pathway were detected in Psa isolates, which are similar to indoleacetic acid (IAA) produced by plant growth-promoting rhizobacteria. No amplicons were obtained for indole-3-acetamide (IAM) pathway genes, a common mechanism for IAA production in *P. syringae* pathovars, however, all isolates harbor *iaaL* gene that encoded for enzyme that produce indole-3 acetyl-3-L-lysine (IAA-Lys), which would have a role in fitness and virulence in others phytopathogens. Presence of 16 effector genes belongs to Biovar 3 cluster, the hyper-virulent pandemic form of Psa, were confirmed in all Chilean Psa isolates. **Conclusion.** In this work, we contrast the high genetic homogeneity between Chilean Psa isolates with phenotypic variation found in virulence factors as biofilm production and bacterial motility. Our results are the first report about the ability of Psa Biovar 3 to produce indole by tryptophan -dependent pathway, however, further studies are needed to determined importance of IAA-Lys in Psa virulence and kiwifruit infections.

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CLP2-42

High-throughput identification of genes required for intracellular survival of *Salmonella enterica* serovar Typhimurium in murine macrophages and *Dictyostelium discoideum*

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Pathogenicity of *Salmonella* is associated to its ability to evade the innate immune system of the host. For instance, *S.* Typhimurium can replicate within phagocytic cells such as macrophages, neutrophils and dendritic cells of many hosts, causing illnesses ranging from gastrointestinal infections to severe systemic diseases. In the environment, *Salmonella* may interact with other professional phagocytic cells, such as free-living amoebae. Amoebas like *Dictyostelium discoideum* ingest bacteria as a source of nutrients and may provide a niche for bacterial replication similar to the intra-macrophage environment. Therefore, we hypothesized that *S.* Typhimurium survival in macrophages and amoebas involves a common set of genes. To identify these genes, we infected RAW264.7 murine macrophages and *D. discoideum* AX4 with a transposon library containing ~200.000 mutants of *S.* Typhimurium 14028s. After co-incubation, extracellular bacteria were killed by gentamicin treatment. Next, intracellular bacteria were recovered at different times of infection (0 and 6 h), grown in LB broth for 12 h, collected by centrifugation and treated for genomic DNA extraction, amplification and massive parallel sequencing. Each mutant was identified through specific barcodes present in the transposon. Analysis of the sequencing data allowed us to identify 720 and 348 mutants under negative selection in murine macrophages and *D. discoideum*, respectively. The comparison of both datasets showed that 213 mutants undergo negative selection in both models. This group includes mutants in genes linked to carbohydrate metabolism and transport (*gnd*, *malP*, *bcsS*), iron uptake (*iroC*, *iroN*, *feoB*), virulence (*hilD*, *hilA*, *sptP*, *sipA*, *sipC*, *invG*, *invF*, *slrP*, *misL*, *mgtB*) and lipopolysaccharide biosynthesis and modification (*waaB*, *waaI*, *waaJ*, *waaL*, *waaZ*, *wbaC*, *manB*, *manC*, *wbaN*, *wbaC*, *wbaD_2*, *oafA*, wzz_{fepE}), among others. Predictions from our high-throughput screenings were confirmed for a selected group of genes linked to LPS biosynthesis and modification using competition assays between defined deletion mutants and the wild-type strain. Altogether, our data indicate that *S.* Typhimurium requires a common set of genes to survive within murine macrophages and *D. discoideum.*

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CLP2-43

Modulation of gut microbiota of rats exposed to binge-like ethanol exposure during adolescence boosted mitochondrial physiology

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Background. An important problem in our society is the consumption of alcohol in adolescence, characterized by drinking of large amounts in a short period. This cause neuroinflammation, oxidative stress and mitochondrial dysfunction. In the present study, we evaluated the effect of dietary intake of *Lactobacillus rhamnosus GG* (*LrhGG*) on the mitochondrial physiology of pre-exposed (BEP) and non-pre-exposed (SP) rats to binge-like ethanol exposure during adolescence. **Methods.** Intraperitoneal injection of either ethanol (BEP) or isotonic saline (SP) were administered to Sprague-Dawley rats at postnatal day 30, 31, 34, 35, 38, 39, 42 and 43. After this, rats was given *LrhGG* orally, and its microbiota was analyzed by qPCR in feces collected after *LrhGG* administration. Then, mitochondrial function was analyzed in livers. Briefly, transcriptional regulation of the mitochondrial protein HIG2A, the antioxidant enzyme MnSOD1 and AMP-activated protein kinase (AMPKα1) was analyzed by RT-qPCR. Moreover, were analyzed the mitochondrial antioxidant capacity, ADP/ATP Ratio and NADH levels. Finally, the levels of respiratory complexes and HIG2A proteins were evaluated by western blot. **Results.** *LrhGG* was found in feces from rats that consumed this bacteria at levels 10^5-10^7 UFC and in the placebo group was undetectable. In SP rats fed with *LrhGG* were induced a significant increase in the expression of the *Higd2a* gene. BEP rats fed with *LrhGG* showed a significant increase in expression of *MnSOD1* and *AMPKα1* genes. Mitochondrial NADH is an indicator of cellular respiration. Its levels in SP rats fed with *LrhGG* were increased significantly. Mitochondrial total antioxidant capacity showed a significant increase in BEP rats fed with *LrhGG*. Besides, the ADP/ATP ratio showed a significant decrease in SP rats fed with *LrhGG.* This last result suggests an increase in cellular proliferation. Finally, *LrhGG* induced an increase of HIG2A protein expression and a decrease of all the respiratory complexes (CI, CII, CIII, CIV and CV). **Conclusion**. *LrhGG* modulates the mitochondrial physiology of rats exposed to binge-like ethanol exposure during adolescence. *LrhGG* likely exerts its beneficial effects through modulation of antioxidant defense and AMPKα1 activation. Changes in the expression of HIG2A and respiratory complexes suggest remodelation of respirasomes.

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CLP2-44

Persistent *Salmonella enterica* serovar Typhimurium infection promotes chronic intestinal inflammation in susceptible mice

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Inflammatory Bowel Diseases (IBD) are chronic intestinal immune disorders that include Crohn's disease and Ulcerative colitis. IBD are the result of an abnormal immune response in susceptible hosts, triggered by a genetic and environmental component. For these reason, several reports have described that infection with enteropathogenic bacteria, such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), could be a risk factor for these diseases. To evaluate the effect of *S.* Typhimurium infection on the onset of IBD, we evaluated in genetic mice model the IL-10$^{-/-}$ mice, and in a chemical, induce by Dextran Sulfate Sodium in wild type mice. Both models were intragastrically infected with *S.* Typhimurium Wild Type (WT) and were treated with antibiotics to clear the bacterial infection for three weeks. Inflammation was evaluated in the intestine and persistence of the bacteria in different organs at the end of the experiment. We found that mice infected with *S.* Typhimurium showed increased levels of inflammation in ileum and colon as compared to non-infected mice. In addition, we detected persistent *Salmonella* infection in different organs of IL-10$^{-/-}$ mice after 42 days of infection, despite antibiotic treatment which we were unable to detect in colitis induction by DSS. Our results suggest that persistent *S.* Typhimurium infection could promotes chronic inflammation in the intestine in a genetic mice model.

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CLP2-45

Cyclovirus detection in feces from Chilean children and adults by real-time PCR

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Background. Cyclovirus (CyCV) is a member of recently proposed genus of the *Circoviridae* family, which includes naked viruses with a small circular DNA detected in insects, birds and mammals. It was first detected in humans in 2010 and it has been reported in 7.3-15.8% feces from children with acute flaccid paralysis (AFP) and 0.73-17.1% in asymptomatic ones. Its pathogenic role is unknown. Infection frequency in feces from Chilean patients has not been studied. The aim of this study was to detect CyCV in feces from Chilean adults and children with neurologic or digestive disease. **Methods.** To this date, 69/180 children and 15/42 adults hospitalized with acute flaccid paralysis (AFP) between 2013 and 2015 from across the country have been studied. Also, 6/16 infants with digestive symptoms from Santiago, Chile were analyzed. Total nucleic acids were extracted using Favorprep® I (Favorgene®) and a fragment of rep protein gene of CyCV was amplified by real time polymerase chain reaction, using primers and probes designed by Beacon Designer 8.0® and the Kapa PROBE® kit in a RotorGene6000®. **Results.** CyCV was detected in 3/69 (4.3%) children with AFP and 2/6 (33.3%) infants with digestive symptoms. C_t (*cycle threshold*) range was 30.8-38.4 cycles. None adult with AFP was positive. Median age (range) and male ratio from each group were: 7 years (2 months - 15 years) and 59.4%; 14.5 months (9 – 33 months) and 83.3% and 54 years (30-74 years) and 75%, respectively. Children with CyCV were three 3 girls with median age of 4 years and from Antofagasta, Rancagua and Concepción. **Conclusion**. This is the first report of CyCV in feces from Chilean patients with neurologic or digestive pathologies. Frequency detected is in accordance with previous international reports. Positive cases are distributed throughout Chile. It is of interest to explore the pathogenic role of this new virus in digestive and neurological diseases.

CLP2-46

Salmonella Enteritidis in poultry meat and meal: a characterization by PFGE

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Background. The frequent presence of *Salmonella* Enteritidis in poultry products and sub-products is a serious hazard for the Chilean exportations. As a response to this risk the industry must have a permanent monitoring based on HACCP plans. The results allow not only to isolate the pathogens but to define where and when this contamination occur. **Design.** We evaluated a set of 38 isolates of *Salmonella* Enteritidis obtained from poultry samples received during 2011 in the Microbiology Laboratory at the Institute of nutrition and Food Technology. The genetic characterization of strains, by means of Pulsed Field Gel Electrophoresis (PFGE), can be used to trace the origin of bacterial biofilms, a key information to implement better control strategy. **Methods.** Salmonella PFGE was performed according to the PulseNET methodology for *Salmonella* Enteritidis. Chromosomal DNA was digested with the restriction endonuclease *XbaI.* The resulting fingerprints were grouped into clusters according to their genetic similarity (≥90% Dice similarity). **Results.** 38 *Salmonella* Enteritidis were grouped into 18 cluster, from which 10 clusters included isolates with a unique genotype and 8 included between 2 or 5 isolates with similar DNA fingerprint. Clusters formed by 2 or more *Salmonella*, grouped isolates from the same batch of product or from different batches processed in close dates, or even different products (meat and meal) processed on different times. **Conclusions.** 1. The results obtained showed that *Salmonella* Enteritidis, with the same DNA fingerprint, could be isolated from 2 different poultry products (meat and meal), suggesting a common origin of contamination. 2. *Salmonella* Enteritidis, with the same DNA fingerprint, from products processed in different dates, suggest the presence of biofilms in the processing line or in poultry farming. 3. The presence of *Salmonella* Enteritidis in poultry meal for animal consumption is of concern, as it contaminates the intestine of another farm animal, increasing the risk of subsequent contamination of meat for human consumption. 4. The industry apply HACCP but these results cannot assure the absence of *Salmonella* in some products.

CLP2-47

Porphyromonas gingivalis AgO contributes to TLR4 increase in cell surface, production of proinflammatory cytokine and migration of OKF6/TERT2 cells

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Periodontitis is a highly prevalent chronic inflammatory disease in Chile and worldwide that mainly affects the teeth supporting tissues. This disease is associated with an infectious process caused by a change in the local ecology of the subgingival biofilm. It has been proposed that the bacterium Porphyromonas gingivalis (Pg) play a key role in the disease onset and progression since induces a dysbiosis that contributes to the inflammatory process. In a previous study from our group, we observed that the O antigen (AgO) region of the lipopolysaccharide contributes to the apoptosis inhibition induced by Pg in epithelial cells, which correlates with an increase in the expression of TLR4. It is known that TLR4 activation increases the production of pro-inflammatory cytokines, and this in turn increase in migratory capacity of human gingival epithelial cells (HGEC), which favors the formation of periodontal pockets. According to this, the aim of this work was to determine whether Pg AgO induce TLR4 activation and if is related to an increase in pro-inflammatory cytokines (TNF-α, IL-8 e IL-1β) and migration of gingival epithelial cells. To address this objective, OKF6/TERT2 gingival epithelial cells were infected at MOI 100 using a reference strain (W50), a mutant lacking the AgO (PG1051) or the complemented strain (CPG1051). After infection, the presence of TLR4 on the cell surface was measured by flow cytometry. In addition, the secretion of TNF-α, IL-8 and IL-1β was quantified by Luminex Multiplex assay. Finally, the migration was evaluated by transwell migration assay. Three independent experiments were performed for each assay (N=3 in duplicate). Our results showed that infection with the strains that possess the AgO region increases TLR4 at the cell surface and the production of pro-inflammatory cytokines ($p<00.5$). This is associated with an increase in cell migration. Our findings suggest, that Pg AgO is required for the activation of TLR4 which would lead cells to have a more migratory phenotype which would be associated to the progression of periodontitis and even could be related to cellular transformation.

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CLP2-48

Development and validation of a real-time Multiplex RT-PCR assay for the detection of Porcine Reproductive and Respiratory Syndrome and Influenza virus in swine

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The porcine respiratory disease complex (PRDC) is a multifactorial disease characterized by a respiratory syndrome and poor growth in fattening swine, which impact the economy in the swine industry. It has been reported that infections or coinfections with the virus of the Porcine Reproductive and Respiratory Syndrome (PRRS) and Influenza A virus (IAV) play an important role in the development of PRDC. Despite the relevance of PRRS and IAV in the disease, there are no commercial kits that allow the simultaneous detection of both pathogens. In this work, we developed a Multiplex real-time polymerase chain reaction coupled to reverse transcription (M-RT-PCR) assay by designing primers sets and hydrolysis probes for the simultaneous detection of PRRS and IAV in oral fluid (OF) and nasal swabs (NS) samples of swine. The results show that primers and probes hybridize specifically to the expected PRRS and IAV target sites, respectively, confirming its specificity and we also ruled out the presence of false positives. Also, it was demonstrated that the Multiplex assay has highly sensitive detecting at least 10 and 100 IAV viral plaque forming units /ml or 10 PRRS RNA copies/ml. Finally, to validate the assay with clinical samples, the RT-PCR Multiplex assay was performed using 803 (657 NS and 146 OF) field samples obtained from swine from different porcine farms of Chile. We found that 14 samples were positive for PRRS, 93 samples were positive for IAV and 3 samples were positive for both viruses. In conclusion, this new Multiplex test can detect simultaneously both pathogens in OF and NS clinical samples, confirming the value of having an assay that provides a differential and the timely diagnosis of these two important pathogens for the porcine industry in Chile.

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CLP2-49

Genes related to membrane biosynthesis are down regulated in response to exposure of *Listeria monocytogenes* at low temperature and copper

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Background. *Listeria monocytogenes* (*Lm*) is an ubiquitous microorganism. In humans, this bacterium causes listeriosis via consumption of contaminated food. *Lm* has the ability to grow at refrigeration temperature, strategy used in food industry to avoid bacteria proliferation. Copper was recognized such as an antibacterial agent in 2008 by EPA, and may be a potential tool to control pathogenic bacteria as *Lm*. Our previous study showed that Cu (sublethal concentration), in combination with low temperature, produce a synergistic effect over *Lm*, decreasing the growth rate. The aim of this study is to identify the main transcriptional changes in *Lm* when it is grown at a low temperature and acutely expose to copper. **Methods.** *Lm* strain List 2-2 was selected for global gene expression analysis by one-channel microarray. Experimental conditions: bacteria growth at 8ºC (control 37ºC) and addition of 0.5 mM of $CuSO_4x5H_2O$ by 1 hour (control media without copper). Data were normalized by quantile and analyzed using Limma package from R statistical software. Data were accepted such as differentially expressed with a B-statistics >90%. **Results.** In the presence of copper, the List 2-2 differentially expressed a total of 263 genes when grown at 8ºC, and 75 genes when grown at 37ºC. The membrane biosynthesis function was the category that included a higher number of genes that changes their expression (13% of genes). Additionally, we observed that genes involved in the transport and metabolism of amino acids and carbohydrates were also modified showing a tendency to repression. Another interesting function affected was cell motility, genes which were significantly down-regulated. Finally, we identified 11 genes differentially expressed only by the effect of copper (not temperature effect). This group of genes included the three genes involved in copper homeostasis (operonZA), which in this case were up-regulated. **Conclusion.** These results showed an early response an acute stress by copper. We presented evidence of a strong down-regulation of genes associated to membrane biosynthesis, metabolism of carbohydrates and flagella biosynthesis, which is most likely due to energy preservation efforts that allow bacterium survival. This research gives insight into use of copper to control contamination by *Lm*.

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CLP2-50

Effect of n-butyrate on the immune response in salmonids cells

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Interplay between microbiota and host is achieved by a chemical cross-talk between molecules secreted by both sides. This cross-talk increase immune tolerance to commensal microbiota, promoting an anti-inflammatory state. The commensal microbiota also help to prepare immune system to respond properly to bacterial and viral pathogens calibrating the cellular response. In Atlantic salmon, the physiological role of its microbiota remains unknown, however the functional equivalence between the mammalians and zebra fish microbiota suggest that in Atlantic salmon, microbiota should interacts with immune system by similar mechanisms. In mammals, the microbial fermentation product Butyrate is a key mediator of this cross-talk, increasing the expression of antiinflammatory cytokines. We hypothetized that Atlantic salmon cells are able to sense butyrate modifiying the production of cytokines and its antiviral response. Using SHK-1 cultures exposed to butyrate (1, 2 and 4 mM) for 24 hours and evaluating by RT-qPCR the expression of several cytokines we show that *in vitro*, butyrate modifies the antiviral response, delaying and reducing the expression Interferon alpha, Mx and PKR. Butyrate also decreases the expression of IL-1β, and induces the expression of IL-10 and TGFβ in agreement with the anti-inflammatory properties reported in mammalians. Interestingly, butyrate also increases the expression of IL-6, a cytokine secreted in response to bacterial infections. Our results suggest that the response of Atlantic salmon immune system is modified by the microbial activity. This implies that factors that modify the composition of microbiota such antibiotics should have important effect over the capacity of the Atlantic salmon immune system to control bacterial and viral pathogens.

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CLP2-51

Detection of intracellular *Helicobacter pylori* in yeasts isolated from the oral cavity and from vaginal discharge

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Background. *Helicobacter pylori* is the causative agent of several gastric pathologies and also the main risk factor to develop gastric. There is evidence demonstrating that this bacterium behaves as a facultative intracellular microorganism in eukaryotic cells, including gastric epithelial cells and macrophages. Besides, recent studies have shown the presence of intracellular *H. pylori* in fungi of genus *Candida,* suggesting that these yeasts could provide protection and help the transmission of this bacterium. Considering the above, the presence of intracellular *H. pylori* in yeasts obtained from the oral cavity and vagina of different groups of individuals of the city of Concepción (Chile) was investigated. **Methods.** Vaginal discharge samples were obtained from the fornix of term pregnant women and oral samples were obtained, using cotton swabs, from students of the University of Concepcion. Yeasts isolated from both types of samples were identified using the following techniques: seeding in CHROMagar *Candida,* germinal tube test and API *Candida,* identification system and later on yeasts were observed by optic microscopy looking for the presence of intravacuolar inclusions followed by immunofluorescence using FITC labeled anti-*H. pylori* policlonal antibodies to verify that the inclusions corresponded to *H. pylori*. Additional confirmation was performed by PCR of the total DNA extracted from all yeasts isolated amplifying *H. pylori* specific genes. **Results.** From a total of 102 samples of vaginal discharge, yeasts were isolated in 44 of them from which 50% were positive for *H. pylori.* On the other hand, from 72 samples of oral cavity, yeasts were present in 24 of them from which 33% were positive for *H. pylori*. **Conclusion.** The presence of intracellular *H. pylori* within yeasts isolated from the oral cavity and vagina of the population under study was demonstrated; thus, reinfection and dissemination of this microorganism could be favored and influence its high prevalence in Chile.

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CLP2-52

Biofilm analysis of *Piscirickettsia salmonis* under different culture conditions

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Piscirickettsia salmonis is the etiological agent of piscirickettsiosis a severe disease that has caused major economic losses in the aquaculture industry since its appearance in 1989. This pathogen is a Gram negative bacterium, facultative intracellular, non-capsulated, non-motile, and pleomorphic. It was recently described that *P. salmonis* can produce biofilm as a persistence and survival strategy in adverse conditions. However, the characterization of culture conditions inducing the biofilm production in *P. salmonis* has not yet been described. The aim of this study is to define culture conditions for the proper induction of biofilm formation in *P. salmonis* strain LF-89. The bacterium was grown under different culture conditions of ferric citrate (0.025, 0.2 and 0.4 mM) and pH (4.80, 7.02 and 8.40) for eight days on 96 wells plates. Growth was measured by determining optical density at 620 nm every 24 h and biofilm formation was quantify using the crystal violet assay. For the assay, biofilm was fixed with methanol, stained with crystal violet and optical density at 595 nm was measured. It was observed that at pH values of 4.8 and 8.4, the bacterium had lower growth rates but higher biofilm production compared to the normal pH of the medium 7.02. For ferric citrate, an increment in the bacterial growth was registered together with the decrease of the concentration of the compound, noticing that the higher biofilm production was at 0.4 mM of ferric citrate from day 5. At the moment, our preliminary results are suggesting that *P. salmonis* strain LF-89 could grow under unfavorable conditions due to the biofilm production, such as low Fe^{+2} concentrations (ferric citrate) and different pH values, such as the ones that are found in the digestive tract of infected fish from estuary or seawaters.

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CLP2-53

Communication between *Bifidobacterium dentium* and *Lactobacillus casei:* perspectives of the interaction in dental biofilm

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Background. Dental caries, a chronic disease with high prevalence in humans, has been associated with the presence of different acidogenics bacteria in dental plaque, a structurally and functionally organized biofilm. Among them, *L. casei* has been found consistently in co-ocurrence with *B. dentium* in deep caries sites. Bacteria in dental plaque can interact and communicate through excretion and detection of signal molecules to coordinate multicellular behavior. This process is known as *quorum sensing*, which plays an important role in initial adherence as well as in biofilm formation. We recently found that both microorganisms produce autoinducer 2 (AI-2), an inter-convertible cell-cell signal molecule spontaneously derived from 4,5–dihydroxy-2,3-pentanedione (DPD) and known as a universal signal for interspecies communication. The aim of this work was determine the effect of AI-2 and cell free supernatant (CFS) of *B. dentium* on adhesion and biofilm formation of *L. casei.* **Methods.** For adherence assay, *L. casei* ATCC 4646 were mixed with saliva and added to a 96 wells polystyrene plate. Plates were culture for 16 hours and attached cells were determined with MTT assay. Chemical DPD and CFS of *B. dentium* ATCC 27534 were used to evaluate its effect on *L. casei* adherence. For biofilm assay, coating slides in saliva were cultured in same conditions described in adherence assay, with time of incubation of 4 days. Biofilm formation was documented with SEM (JSM-IT300). **Results.** *L. casei* ATCC 4646 co-culture with chemical DPD exhibits better adherence after 16 hours, compared with culture with no external source of DPD. Furthermore, in biofilm assay, also exhibit increased biofilm formation. Same effect was seen when CFS from cultures of *B. dentium* ATCC 27534 were added in adherence assay and biofilm formation. **Conclusion.** Our data supports the idea that the presence of AI-2 increases adhesion and biofilm formation of *L. casei.* We propose that the effect observed in *L. casei* by CFS of *B. dentium* is related with AI-2, suggesting that the presence of *B. dentium* could potentiate *L. casei* development. Further investigations will be needed to determinate if AI-2 acts as an interspecies signal among them in dental biofilm.

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POSTER SESSION III

CLP3-1

Identification and characterization of ROS-independent tellurite targets in *Escherichia coli*

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Background. The oxyanion tellurite is highly toxic to most organisms, including bacteria. To date, most studies aimed at elucidating the mechanisms of toxicity and intracellular tellurite targets mainly in aerobic conditions. Once inside the cell, tellurite oxidizes sulfhydryl groups of proteins and antioxidant compounds such as glutathione, which is hypothesized to result in the generation of superoxide radical and other reactive oxygen species (ROS). For this reason, it is thought that oxidative stress is the major responsible for tellurite's toxicity. However, recent studies from our laboratory showed that there is no correlation between increased tellurite and hydrogen peroxide or superoxide resistance, suggesting that tellurite is toxic *per se*. The current study aims to identify those molecules, genes, metabolic pathways, etc., affected by tellurite in the absence of ROS. **Methods.** All experiments were conducted using *E. coli* BW25113 cultures grown in minimal M9 medium in anaerobiosis. Tellurite entrance was determined by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy). The genes involved in tellurite resistance were identified by chemical genomics analysis using a pool of 6000 *E. coli* deletion mutants coupled with high throughput sequencing. As a complementary strategy, we generated highly tellurite-resistant *E. coli* strains by directed evolution under anaerobic conditions. Using several biochemical techniques, we identified macromolecules directly damaged by tellurite. **Results.** In anaerobiosis, the tellurite minimum inhibitory concentration for *E. coli* increased 100-fold as compared to aerobic conditions. Chemical genomic profiling showed that tellurite affected several metabolic pathways including the Krebs cycle, phospholipid synthesis, and synthesis and degradation of amino acids, ribo- and deoxyribonucleotides. **Conclusion.** *E. coli* tolerates greater amounts of tellurite under anaerobic conditions, which remain low as compared to other oxyanions. Our results suggest that the damage generated by tellurite is related to nucleotide and amino acid depletion, thus impeding that *E. coli* can repair and survive the stress imposed by this oxyanion. This work is part of the first steps to find and identify the targets of tellurite independent of ROS.

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CLP3-2

Antimicrobial-resistant *Salmonella enterica* strains belonging to zoonotic serotypes in dairy farms from the Metropolitan Region, Chile

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In human populations, infection with *Salmonella enterica* represents the most common outbreaks of any infectious disease in the last 30 years. It includes many serotypes, from which currently the highest incidences at national and international level are represented by *S.* Enteritidis and *S*. Typhimurium. Infection in humans frequently occurs by consumption of animal-derived foods, or by direct contact with domestic animals. The aim of this work is to describe the presence of *Salmonella* serotypes in dairy farms from the Metropolitan Region. For this purpose, 566 feces samples from the pens environment were collected in two farms: farm A with 306 samples, and farm B with 260 samples. *Salmonella* isolation was performed under standard procedures. Then, isolates were serotyped according to the Kaufmann-White scheme, and the phenotypic resistance to antimicrobials was determined by the disk diffusion method. Statistical analyses were performed through 2x2 contingency tables. As results, 44 *Salmonella* strains belonging to serotypes Typhimurium (17), Livingstone (24) and Infantis (3) were isolated. Among them, eight antimicrobial-resistance patterns were detected, some of which being significantly associated to *S*. Typhimurium isolates ($p<0,05$). In fact, all strains belonging to this serotype were resistant to at least one antimicrobial, in contrast to *S*. Infantis and *S*. Livingstone, in which 100% and 63% of their isolates were pansusceptible. In addition, all *S.* Typhimurium strains were isolated from farm A, and all *S*. Infantis and *S*. Livingstone strains were isolated from farm B. According to data from Instituto de Salud Pública (ISP), these three serotypes are among the top ten serotypes isolated from human clinical cases in Chile. Although genotypic analyses should be performed to determine the epidemiological links between bacteria isolated in dairy farms and human disease, the presence of resistance phenotypes against critically important (cefotaxime, ciprofloxacin, enrofloxacin) and highly important (chloramphenicol, tetracycline) antimicrobials, as defined by WHO, suggests that foods derived from cattle might represent public health risks. The pathogenic potential of these isolates and their transmission cycles in animal farms are remaining questions which, once responded, will support control measures and clarify the associated sanitary risks.

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CLP3-3

Bioprospecting marine actinobacteria from Easter Island

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Antimicrobial resistance has become troublesome affecting the population worldwide, resulting in the reduced efficacy of antibiotics used in the treatment of infectious diseases, creating an urgent need for the search of new antimicrobial agents. Unexplored habitats enhance the probability of finding new bacterial strains that can lead to the discovering of novel secondary metabolites. Usually, studies are focused on strains from the *phylum Actinobacteria*, due to the considerable evidence of bioactive secondary metabolites produced by strains of this *phylum*. In this sense, this research aims to isolate marine actinobacteria from marine sediment and sponge samples obtained from the Motu Nui Islet, Ovahe beach and Terevaka beach, all located in Easter Island, Chile, places considered to have an unexplored microbial biodiversity.

The isolation process and comparison of 16S rRNA gene sequences led to the identification of 82 actinobacteria belonging to 13 genera: *Arthrobacter*, *Brachybacterium*, *Brevibacterium*, *Gordonia, Knoellia*, *Kocuria*, *Kytococcus*, *Micrococcus*, *Nesterenkonia*, *Nocardioides, Rhodococcus, Serinicoccus* and *Streptomyces*. To study the biotechnological potential for producing biologically active compounds, antimicrobial activity tests were accomplished against 5 bacterial model strains of clinical interest: *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica*. An initial screening using antibiograms were accomplished, where inhibitions against model strains were produced by 14 actinobacteria belonging to the genera: *Brachybacterium, Brevibacterium, Gordonia, Kocuria, Kytococcus, Micrococcus, Nocardioides* and *Streptomyces*. These strains were subsequently evaluated for antimicrobial activity testing with crude extracts, where the results showed that 10 strains presented inhibitions against *Staphylococcus aureus*, and 3 strains also inhibited *Listeria monocytogenes*. Then, 11 strains were tested for the detection of polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) biosynthetic genes, which are involved in the synthesis of bioactive compounds. The results show that *Brevibacterium, Kocuria, Kytococcus, Nocardioides* and *Streptomyces* strains presented at least one of the genes that codify for PKS-I, PKS-II or NRPS, while *Brachybacterium* strains did not present positive results for any of the three cases. This study reveals that a diverse actinobacterial biodiversity exists in marine sediment and sponge samples of Easter Island, Chile, including strains that have a great potential for producing bioactive compounds.

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CLP3-4

SCCmecCLA; a machine learning approach for the identification, classification and visualization of *Staphylococcal* cassette chromosome *mec*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of infection in human populations worldwide. An important feature of this organism is the presence of a specific genomic island denominated Staphylococcal Cassette Chromosome *mec* (SCC*mec*), which contains the proteins responsable of its resistance against β-Lactam antibiotics. The length of this island goes from 20,000 base pairs to 80,000 depending on the SCC*mec* type (I-XI). To carry out the classification and typing of the SCC*mec* element, the structure of two regions within the cassette are important: the *mec* complex; the region behind the resistance and the set of recombinasas; proteins responsible for mobility by cleavage and integration. Based on this classification scheme, 11 different genetic organizations of the *mec* complex and different allelic patterns in the recombinases have been described, along with variants often within the same type determined by the presence of new genes. The typification of SCC*mec* is usually done by PCR, using primers specific for different regions within the cassette. However, several limitations to this process exists, including differences in the classification result according to the origin of the set of primers used, and the labor required to carry out the typification for multiple strains in the lab. In addition, it limits the classification of new variants that can not be detected by the set of primers already described. With this in mind, we developed an *in silico* tool for the classification and visualization of SCC*mec* from *S. aureus* genomic sequences, at the contig level for classification and visualization and at the sequence read level for classification. This tool is based on the use of two complementary methods. By using k-mers to create a distance based network and through automatized cluster analysis we were able to not only classifiy current SCC*mec* types, but also detect novel types in publicly available genome databases. The second approach consists in the implementation of a robust machine learning algorithm, *k*-Nearest Neighbors algorithm (*k*-NN), used to determine whether analyzed sequences corresponds to one of the SCC*mec* type already described.

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CLP3-5

High prevalence of class 1 Integrons in different multidrug-resistant bacteria isolated from *Salmo salar* microbiota and seawater in Chilean salmon farming

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One of the most important issue in Chilean salmon farming is the high use of antibacterial agents to control of infectious diseases. Tons of antibiotics are used every year, where the most used antibacterial agents in this industry are oxytetracycline and florfenicol. It's well known antibiotics are able to modify natural bacterial communities, selecting resistant microorganisms and antibiotic resistance genes. The spread of antimicrobial resistance in bacteria is a complex process involving a diversity of different mechanisms. Currently, one of the most important genetic element involved in the dissemination and recruitment antibiotic resistance genes are Integrons. It's a versatile gene acquisition system commonly found in bacterial genomes. These elements are able to acquire open reading frames embedded in exogenous gene cassettes and convert them to functional genes by ensuring their correct expression. They can be transferred between bacteria by transposons or plasmids in which they are present. Integrons have been widely studied in pathogenic bacteria but little is known the role are playing in water environment as salmon farming in the south of Chile. In this work we have analyzed different class of Integrons and antibiotic resistance genes of 68 different multidrug-resistant bacterial isolates from *Salmo Salar* microbiota and seawater. We characterized 12 different resistant bacterial genera from microbiota and seawater, and we have been examining cross resistance to different families of antibiotics. Our results show class 1 Integrons are high prevalent and widely distributed in Chilean salmon farming since it was found in all isolates. Furthermore, we found different antibiotic resistance genes inside integrons as aminoglycosyltransferases, dihydrofolate reductases and beta-lactamases, apart typical *sul1* and *qacEΔ1* genes. These genes were found in *Pseudomonas, Serratia, Aeromonas, Brochothrix, Shewanella* and *Pseudoalteromonas.* Moreover, our results show a high multi-resistant to different families of antibiotics where a high proportion of isolates are resistant to tetracyclines, chloramphenicols, macrolides and polymyxin. These results suggest class 1 integrons are playing an important role in dissemination and recruitment antibiotic resistance genes of different antibiotic families in Chilean salmon farming, and the high use of antibiotic has selected resistant bacteria and antibiotic resistance genes in water environment.

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CLP3-6

Evaluation of microalgae extracts as potential natural Trypanocidal drugs

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Background. Chagas disease is a neglected parasitic infection, caused by the protozoan *Trypanosoma cruzi*,that constitutes a Public Health problem in Latin America, affecting more than 8 million people worldwide, including Chile. Nifurtimox (Nx) and Benznidazole (Bz) are the only available drugs for the treatment of this disease. Nevertheless, they present various adverse effects as well as low efficiency in the chronic phase of the disease. In this context, there is a growing interest about the search for natural anti-chagasic compounds, whose origin could be in plants, macroalgae or microalgae. Microalgae are single-cell photosynthetic eukaryotic organisms, which present different types of compounds that possess biological activity, such as antioxidant and anti-inflammatory activity as well as and cytotoxic activity against neoplastic cells and pathogen. The aim of the present study was to evaluate the trypanocidal activity of different extracts from microalgae and cyanobacteria against the infective cellular form of the parasite. **Methods**. Ethanolic, methanolic and aqueous extracts from the microalgae *Tetraselmis suecica*, *Scenedesmus obliquus, Nannochloropsis oculata, Chlamydomonas reinhardtii* as well as from the cyanobacteria *Arthrospira platensis* were obtained. The trypanocidal effect as well as cytotoxicity in mammalian cells of the extracts were analyzed by the MTT method. Additionally, the cytotoxicity of the most promising extracts was evaluated in combination with Nx. **Results**. Four microalgae extracts showed trypanocidal activity with an IC50 lower than 100 ug/ml: Methanolic extracts of *S. obliquus* and *T. suecica*, and ethanolic extracts of *C. reinhardtii* and *T. suecica*. Importantly, no cytotoxic effects were observed for those extracts for mammalian cells. **Discussion and Conclusions**. Our results suggest that methanolic extracts of *T. suecica* and *S. obliquus* as well as the ethanolic extracts of *C. reinhardtii* and *T. suecica*, are promising potential candidates for the development of future anti-chagasic drugs.

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CLP3-7

Study of the production of extracellular polymeric substances in the filamentous cyanobacteria *Trichormus* sp.

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Cyanobacteria are photoautotrophic prokaryote organisms that are unicellular or filamentous. Many species of cyanobacteria can produce extracellular polymeric substances (EPS), which may be bound to the cells or released into the extracellular medium (R-EPS). The EPS are constituted by a structural backbone of carbohydrates with anionic nature due to the presence of sulfonic and uronic groups. The EPS allow cell aggregation and filament formation, giving cyanobacteria the ability to adhere to various substrates, such as soil. We isolated eight strains of cyanobacteria from soil samples of different Chilean Regions, and the relative production of R-EPS was analysed on each strain. The cyanobacteria *Trichormus* sp. was the only axenic culture and showed the highest content of R-EPS with a value of 55.5 mg on the total volume culture. The growth curve of *Trichormus* sp. was characterized and the production of R-EPS was determined at different phases of growth, up to 30 days. It was observed that the highest production of R-EPS occurs during the stationary phase, between 14 and 20 days. The preliminary characterization of the fraction of R-EPS of *Trichormus* sp. indicated the presence of anionic functional groups, representative of cyanobacterial EPS. This is a pioneering work in Chile in the study of native cyanobacteria and R-EPS production with biotechnological perspectives.

FONDEF IT16I10090 Desarrollo de una cobertura compuesta para el control de erosión eólica en depósitos de relave

CLP3-8

Heterologous expression in *Escherichia coli* of a psychrophilic subtilisin-like enzyme from an antarctic isolate of *Polaribacter* sp.

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Peptidases, also known as proteases, are enzymes with hydrolytic activity that degrade proteins, and they are of interest in cosmetic, textile and food processing industries. Subtilisins are serine-proteases belonging to the S8 peptidase family, which have been widely used as additives in detergent formulations. Subtilisins used industrially come from bacteria, and in some cases their parameters of optimal activity have been modified by protein engineering to decrease the optimal temperature of activity. A distinct source of these enzymes with high activity at low temperatures are antarctic microorganisms. Production of a cold adapted subtilisin-like enzyme was found in an antarctic isolate of *Polaribacter sp*. and it was previously characterized. Obtaining this enzyme from the Antarctic microorganism requires at least 48 h of culture at 4 °C. The aim of this work is to implement a more efficient recombinant production system for this psychrophilic subtilisin. The coding sequence of this enzyme was optimized according to the codon usage of *Escherichia coli* and it was heterologously expressed in this bacterium given the advantages in induction time, amount of recombinant protein produced and the well known culture conditions. Recombinant proteases could affect bacterial growth, so the cloned sequence of subtilisin corresponds to the inactive pro-protein. After intracellular induction of the recombinant pro-protein, subtilisin activity was measured on a chromogenic substrate. The unpurified enzyme had an activation process that takes several hours, suggesting that in the intracellular medium it remains as pro-protein. Thus, the psychrophilic subtilisin is produced as an inactive recombinant enzyme in *E. coli*, and it shows a slow activation process. This result would facilitate the process of modifying the enzyme by mutagenesis to improve such characteristics as oxidation resistance or thermostability.

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CLP3-9

Rewiring the metabolic network of *Pseudomonas putida* KT2440 for the production of tailor-made polyhydroxyalkanoates (PHAs) on glycerol

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Polyhydroxyalkanoates are linear oxopolyesters that can be synthesized by numerous bacteria as inclusion bodies in the cytoplasm of the cell, providing a form of energy and carbon storage material. It is promoted in the cell when there is a high concentration of carbon substrate accompanied by the limitation of an inorganic nutrient. *Pseudomonas putida* strain has been a well-studied natural producer of medium-chain-length (mcl) polyhydroxyalkanoate (PHA) containing C6-C14 monomer units from different carbon sources. Microbial polyhydroxyalkanoates (PHA) are a family of biodegradable and biocompatible polyesters which have been extensively studied using synthetic biology and metabolic engineering methods for improving production and for widening its diversity. To synthesize the novel biopolymers on glycerol, we target two important pathways for the synthesis of mcl-PHA when cells are growing on carbohydrates, namely the synthesis *de novo* fatty acids and the reversed β–oxidation route. Knockout mutants *fabD* (malonyl-CoA-ACP transacylase) and *phaG* (Hydroxyacyl-ACP transacetylase) were constructed by using the plasmid pEMG, where the PCR fragments was integrated into pEMG via restriction sites. Furthermore, the pEMG-derivates were co-integrated by a single cross-over into the chromosome of *P. putida* KT2440. The successful genomic deletion of the target open reading frames was finally confirmed by PCR.With the objective of re-directing the carbon flux to obtain specific PHAs, the endogenous *P. putida* KT2440 *fadA* (3-ketoacyl-CoA thiolase) and *atoB* (Acetyl-CoA acetyltransferase) genes were overexpressed by inserting them into IPTG-inducible expression vectors pSEVA424-228. Thus, the cell will preferably use the acetyl-CoA pool for the formation of PHAs. We tested the PHA producing capacity of each created mutant in batch mode using glycerol as the only carbon source. The proposed modifications for strain KT2440 resulted in the redirection of the carbon pool towards the formation of PHAs along with the reduction of the biomass formation, which also produced the structural modifications of the biosynthesized PHAs. Currently, we are characterizing the produced PHAs in terms of monomer composition, molecular weight, and thermal properties (ITC and GS-MS). This is the first report showing the production of specific biopolymers using a natural PHA-producing strain with non-related carbon sources such as glycerol.

Proyecto FONDECYT 11150174

CLP3-10

Isolation and characterization of novel proteobacterial strains having the ability to use furan aldehydes as the only carbon and energy source

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Lignocellulosic biomass is an abundant and renewable source of sugars for the microbial production of fine chemicals and biofuels. However the formation of toxic fermentation inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) during acid pretreatment of lignocellulose imposes a significant technical challenge for an efficient bioprocess. The presence of these furans decreases the growth and productivity of bacterial and yeast strains used for the formation of ethanol and other biochemical products. Consequently microbial metabolism of furanic compounds is rapidly gaining interest in the scientific community having in mind a hypothetical biodetoxification procedure, relying in microorganisms to degrade these inhibitors. However the slow rate of furans biodegradation limits its practical application. Furthermore, limited information about furans biodegradative pathways and their encoding genes is available, mostly due to the fact that very few organisms have been identified which completely metabolize furfural or HMF. In this work we reported the isolation and characterization of fifteen novel proteobacterial strains having the ability to use furfural and/or HMF as the only carbon and energy source. All the strains were isolated from activated sludge treatment of bleached kraft pulp mill wastewater and most of them were classified in the *Pseudomonas* genus according the sequence of their 16S ribosomal RNA-encoding genes. Most remarkable is the fact that several of these strains showed a much more efficient catabolic phenotype for turnover of furfural and HMF, including faster growth and increased tolerance to high substrate concentration, than previously isolated microorganisms. Genome sequencing of these novel strains is currently undergone in order to identify the organization and phylogenetic relationship of the genes encoding the catabolic phenotypes for furfural and HMF. The genetic and biochemical characterization of the furans biodegradative pathways in these strains holds great promises for industrial applications such as the biodetoxification of lignocellulosic hydrolysates and the production of value-added compounds.

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CLP3-11

Global proteomic response of *Acidithiobacillus ferrooxidans* ATCC 53993 to cadmium

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Microorganisms have developed a variety of mechanisms to control heavy metal levels inside the cells. Cadmium does not have a biological function described. Nevertheless, is able to get into the cell through Mn^{+2} and Zn^{+2} transporters, disrupting cellular functions. Some mechanisms to control intracellular metal levels are efflux systems belonging to three families: P-type ATPases, RND transporters and cation diffusion facilitators (CDF). Despite the existence of such systems, microorganisms have low resistance to cadmium, an even low concentrations (μM) of the metal can cause cellular death. However, *Acidithiobacillus ferrooxidans* is an acidophilic bacterium capable of resisting very high concentrations of heavy metals such as cadmium. This is important in mining processes where Cd^{+2} concentrations can be in the range of 5-100 mM. The cadmium resistance mechanisms in acidophilic microorganisms have not been fully characterized. *A. ferrooxidans* strain ATCC 53993 contains possible metal resistance determinants similar to those found in the other acidophilic bacteria and particularly in its exclusive genomic island. These determinants showed functionality when cloned and expressed in *Escherichia coli.* To identify new possible cadmium resistance strategies in *A. ferrooxidans* ATCC 53993, the global protein expression of cells grown in the presence of cadmium was analyzed by using iTRAQ quantitative proteomics. The results showed some metabolic strategies that could contribute to cadmium resistance. Amongst them there are changes in the iron oxidation pathway, upregulation of inorganic ion transport proteins, variations in ribosomal proteins and amino acid biosynthesis pathways.

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CLP3-12

Study of the trajectory of xylose and oxygen uptake rates over the phenotypic phase plane of *Scheffesomyces stipitis* in fed-batch cultures

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Xylose is one of the main constituents of the lignocellulosic material, which can be used as raw material for the production of second generation biofuels. *Scheffersomyces stipitis* is a yeast which relevant feature is its ability to metabolise xylose into ethanol with a high yield and tolerance to ethanol. Its growth profile and ethanol production are dependent on the oxygen dissolved concentration in the media, being only able to produce ethanol under oxygen limited conditions and is not able to grow under anaerobic conditions. Literature provides metabolic models at a genomic scale that allow the exploration of the yeast behaviour, as well as kinetic models which describe its growth and production profile. This work focuses on the determination of the trajectory of the specific uptake rates of oxygen and xylose in *S. stipitis* fed-batch cultures (FBC), on the phenotypic phase plane. This plane is determined using FBA (Flow Balances Analysis) on the microorganism´s genomic scale metabolic model and solved using COBRA (Constrained Based Reconstruction Analysis) software and TomLab / CPLEX optimizer 7.8. Fermentation conditions defining the desired trajectories on the phase plane were obtained using a kinetic model for *S. stipitis* culture linked to FBA using Matlab R2015b software. This are, a constant feed stream, minimum mineral media supplemented with yeast extract and mineral traces at a temperature of 30 °C and pH 5. Stirring was kept within a range of 100 to 350 rpm, and an aeration of 15 to 75 mL min^{-1} were used in the fermenter. Thus, fermentation experiments were carried out in an 0.5 L Applikon MiniBio fermenter, with an initial volume of 0.3 L. Thus, using kinetic models to represent the growth of *S. stipitis*, in combination with metabolic models of genomic scale, it is possible to determine the trajectory on the plane of phenotypic phases that experimentally determine an increase in the production of ethanol from xylose.

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CLP3-13

Influence of the use of native non-*Saccharomyces* yeasts on the aromatic profile of a wine cv Cabernet Sauvignon in co-inoculation with *Saccharomyces cerevisiae*

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The alcoholic fermentation process (AF) in Chile is mainly carried out by the inoculation of strains of commercial yeasts of the genus *Saccharomyces*, which carry out a controlled microbiological fermentation. However, this has led to the standardization of production and to the obtaining of a correct product from the oenological point of view, but which lacks complexity and aromatic typicity of the region of origin. The aim of this research was to use native yeasts non-*Saccharomyces* and to evaluate their contribution in the aromatic profile of a wine cv Cabernet Sauvignon, initially evaluating the native yeast implantation using a PCR-ITS 5.8S rRNA-RFLP strategy, to later evaluate the contribution of native yeasts to the aromatic profile of the wine obtained. Two native non-*Saccharomyces* yeasts, one *Metchnikowia* (T1) and one *Hanseniaspora* (T2) and a control with *Saccharomyces cerevisiae* (EC1118) were evaluated, performing sequential inoculations were carried out starting with the native non-*Saccharomyces* yeasts and after 48h the yeast *Saccharomyces cerevisiae* EC1118. AF was monitored by direct yeast counts and measurement of fermentable sugars and ethanol production. When doing the analysis of the implantation, it was observed that the inoculated native yeasts were found in the initial stages of the AF to finish the process with the detection of only the control yeast. At the end of the AF, the aromatic profile was evaluated using the HS-SPME method through a mass-gas chromatograph, analyzing 23 volatile aromatic compounds that were grouped into esters, alcohols, acids, aldehydes and terpenes. The treatments T1 and T2 were the ones that obtained the highest concentration of aromatic compounds, showing statistically significant differences with the control. Within the aromatic groups found we can emphasize the esters, alcohols and terpenes and the increase of these volatile compounds in the wine translate into an increase of the fruity and floral aromas that make of this wine an aromatic product more complex and distinctive. Therefore, we can conclude that the incorporation of non-*Saccharomyces* native yeasts, in *Saccharomyces* co-inoculation on must cv Cabernet Sauvignon increased the aromatic complexity of the wines produced in this study.

Proyecto FONDEF ID14I10010

CLP3-14

Influence of NaCl on the PHA synthesis in *Pseudomonas putida* strains on glycerol

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Background. Conventional plastics are versatile polymers but they cause serious environmental problems due to their persistence in the environment. Microbial synthesis of biopolymers is a sustainable path to petroleum-based plastics. Polyhydroxyalkanoates (PHAs) are biopolymers synthesized by many bacteria as an intracellular reservoir material of carbon and energy that can be mobilizes during famine. Furthermore, they possess thermoplastic and elastomeric properties and have the advantages of being biodegradable, biocompatible, and non-toxic. The production of PHA using the Gram-negative bacteria *Pseudomonas putida* KT2440 has been widely studied, being capable of producing up to 30% of its cell dry weight as biopolymer, when cells are grown on glycerol. As PHA synthesis is a non-growth associated process, nitrogen and phosphorous limitations is imposed in the culture broth to promote PHA accumulation in the cell. PHA synthesis can be also affected by various environmental conditions such as high salt concentrations. *Pseudomonas putida* H has the ability to growth under harsh conditions due to its mobile genetic elements. In this work we will assess the PHA production capacity of both *P. putida* KT2440 and H on increasing NaCl titers using glycerol as carbon source. **Methods.** In this work, we used *P. putida* H *and* KT2440 to produce PHA in batch cultures. For this, the bacteria were grown on glycerol as carbon source in M9 medium at different salt concentrations to assess important physiological parameters and PHA production. **Results.** We found that *Pseudomonas putida* H can cope with a high salt concentration (up to 34 g/L of NaCl), showing a specific growth rate of 0.20 (1/h). On the other hand, *P. putida* KT2440 maintained a specific growth rate of 0.19 (1/h) when grown up to 10 g/L of NaCl. Salt concentrations above this value result in a decrease in the specific growth rate a biomass yield. **Conclusion.** This study shows that *P. putida* H can grow at a high NaCl concentration, where the specific growth rate and biomass yield were unaffected by the imposed conditions. We expect that the PHA content in the cell will be also unaltered, paving the way to an unsterile PHA production system.

PROYECTO FONDECYT 11150174

CLP3-15

Application of metal(oid)-resistant bacteria in remediation

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Background. Metal(loid) contamination is a current environmental issue; for that reason, employing microorganisms and/or their metabolic abilities to remove metal(loid) ions in aquatic systems appear as a plausible option to decrease metal(loid) pollution, through different strategies. Likewise, sorption or bacterial reduction of these metal(loid) species a strategy that allows a change in the oxidation state of the ion thus turning it into a less toxic species. **Methods.** To remove $CdCl_2$, $NaAsO_2$, K_2TeO_3 and $CuSO_4$ from the medium, environmental strains isolated from different locations of the Chilean territory were used. These display metal(loid) resistance as compared to *E. coli*, that is considered as a sensitive specie. As the first approach, the MIC of the metallic salts mentioned above was determined for 16 different environmental strains; the most resistant were selected for further analysis. Then, selected strains were cultured in media containing individual or mixed metal(loid)s. The concentration of metal(loid)s was assessed over time (24 h) and aerobic or anaerobic conditions were used for comparison purposes and to evaluate yield. All parameters were scaled up to a bioreactor to improve the metal(loid) removing process. **Results.** Selected strains were about 200 times more resistant than *E. coli* for tellurite, 3 times for arsenite and no change was observed for cadmium and copper. It was also observed that all four salts mixed were considerably more toxic and inhibited growth even at low concentrations. Taking this into consideration, cultures of individual strains showed over 50% tellurite reduction. For copper and arsenite a similar behaviour was observed, with <50% of removal. Cadmium, was not soluble in the culture medium. Mixed cultures showed decreased yields as compared to individual strains. Finally, only a 10% increase of metal(loid) removal was observed in the batch culture. **Conclusions.** The bioremediation process resulted in higher metal(loid) removal using individual strains, proposing metal(oid)-resistant as a candidate for remediation process.

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CLP3-16

Prospecting for lipolytic bacterial strains for the treatment of wastewater with high oil and grease content generated in the food service industry

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Background. Wastewaters from the food service industry contain high levels of fats and proteins that present low biodegradability. The accumulation of fatty wastewaters produces clogging and the emergence of unpleasant odors. Therefore, the application of enzymatic treatments (i.e. bacterial lipases) is an alternative to improve the biological degradation of fatty wastewaters. The aim of the present study was to perform the first steps to formulate a bacterial consortium with lipolytic activity to ameliorate the treatment of liquid industrial wastes originated in the food service industry. **Methods.** Bacteria were isolated from liquid industrial wastes derived from grills, cafeteria, bakeries and fast food places. Isolates were evaluated for their lipolytic activity in media supplemented with Tween 20 and identified by 16S rRNA sequencing. Lipase activity was measured by a colorimetric method and evaluated at different pH and temperatures. In addition, the structure of the total bacterial communities was studied using DGGE. **Results.** A total of 92 isolates were obtained from the samples. Fifty-two strains exhibited potential lipolytic activity. Sequencing results showed that the isolates belong to the genus *Bacillus, Serratia, Hafnia, Staphylococcus* and *Aeromonas.* High lipolytic activity was observed in six strains and a decrease of about 50% of the enzymatic activity was observed at pHs under and over the optimum (pH = 7). The bacterial lipases showed thermostability at 60°C and 80°C. **Conclusion.** It was possible to generate a collection of bacteria isolated from liquid industrial wastes originated in the food service industry with a high lipolytic activity. Based on these results, a potential lipolytic bacterial consortium could be design.

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CLP3-17

Transcriptomic analysis in a simple consortium of the infant gut microbiome mediated by prebiotics shift in a continuous biorreactor culture

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The gut microbiota has been shown to have a strong impact on host health. Early composition of the gut microbiome is influenced by infant diet, where human milk oligosaccharides (HMO) act as prebiotics, and microbial interactions mediated by infant diet are essential for microbiome assembly. Here we investigated the transcriptomic profile of four representative species of the infant gut microbiome in a controlled anaerobic bioreactor simulating a dietary change. A microbiome consortium composed of *Bifidobacterium longum* subsp. *infantis*, *Bacteroides vulgatus*, *Escherichia coli* and *Lactobacillus acidophilus* was cultured on fructooligosaccharides (FOS) or on 2´-fucosyllactose (2FL). We growth the consortium in batch conditions until reach stationary phase using FOS as carbohydrate source. Then, the bioreactor was switched to a continuous operation. Initially, the OD_{630} and pH remained constant, indicating that the consortium shifted from batch stationary phase to an exponential growth mediated by continuous income of medium. After 12 h growing continuously in FOS, carbon source was switched to 2FL. At this stage, OD_{630} initially decreased, stabilizing toward the end of the experiment, suggesting a phase of adaptation to the carbon source. The abundance of each bacteria across the time was measured by qPCR, showing that *L. acidophilus* dominates the fermentation over the others 3 bacteria. Gene expression was measured by qRT-PCR at 6h, 12h and 18h, showing changes in relative expression of genes related with carbohydrate metabolism such as alpha-L-fucosidase, fructokinase, beta-galactosidase, L-serine ammonia-lyase and galactokinase. Individual carbohydrate consumption was analyzed by Thin Layer Chromatography showing a decrease of FOS after 2FL shift. Protein profile was studied using Bradford assay, indicating no protein consumption or protein based fermentation. Short Chain Fatty Acid (SCFA) production were analyzed by HPLC, showing no production of propionate and butyrate. However, lactate was produced during FOS step, and decrease its concentration after 2FL shift. Conversely, acetate was produced after 2FL shift. Altogether, this results indicates that dietary oligosaccharides differentially modulate microbial behaviour, mediate changes in gene expression and SCFA production regard the HMO used as carbon source. Moreover, this continuous system could be extended to study microbial interactions of microbiome species in a controlled environment.

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CLP3-18

Pollution & Inundation: drivers of changes in the diversity and composition in bacteria of coastal soils in Antarctica

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Hydrocarbon pollution is a global-scale issue. In Antarctica, hydrocarbons are present in soil and seawater due to fossil fuels used in human settlements, where the environmental conditions such as low temperature help the pollution persistence. The objective of this work was to analyze the effect of both hydrocarbon pollution and seawater inundation over the microbial assemblages in Antarctic coastal soils. For this, two polluted and two non-polluted soil sites including one of them flooded by seawater during the high tide were sampled on the shore of Chile Bay, Greenwich Island (South Shetlands). Bacterial community diversity and composition was analyzed by 16S rRNA gene sequencing. The ANOVA was used to test pollution, inundation and phylum as source of variance of the microbial alpha diversity, showing that the effect of each factor depends on the other two. Tukey's test was also carried to assess differences in diversity inside each bacterial phylum as response to pollution and inundation. According with ANOVA results, each phylum was influenced differently by both factors. Furthermore, taxonomic composition showed that pollution effect was notorious, whereas inundation effect was clearer at the genus level. OTUs related to hydrocarbonoclastic bacteria were present exclusively in the polluted samples, including some present in the inundated samples. In conclusion, the effect of pollution is significant on the diversity and microbial composition, and the flood could incorporate some taxa in the coastal soils, whose settlement would depend on the soil conditions.

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CLP3-19

Salt response in the recurrent psychro- and halotolerant *Exiguobacterium* sp. SH31 isolated from sediments of the Salar de Huasco, Atacama Desert

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Extremophiles microorganisms are capable of inhabiting hostile environments such as variation temperatures, pH and salt, UV light exposition and even toxic compounds, as well as Reactive Oxygen Species (ROS) formation and the co-occurrence of several of these events. A *Exiguobacterium* strain was isolated from Salar de Huasco (Atacama Desert), is a well-known shallow lakes area with variable concentrations of salts, little human intervention and extreme environmental conditions which makes it ideal for resistant mechanisms study and evolutive adaptation. This bacterial genus has not been extensively studied, but it has high plasticity and adaptation capacity, evidenced as it is cosmopolitan, however to date there are no studies about tolerance and resistance to salinity. We proposed as hypothesis that *Exiguobacterium* sp. SH31 modulates its genotypic patterns in response to the salt concentrations that it faces. In this context, as a first step to characterize the response to the SH31 strain to salinity and to stablish the bases for a molecular study, we proposed to compare the growth and response to these bacteria in three different salt conditions (0,25 and 50 gr/L NaCl) under different physiology, genomic and transcriptomic approaches. The bacteria growth was analyzed under a temperature range (25 – 42 °C) and concentrations of NaCl (0 – 75 gr/L), so as a result 25°C and 25gr/L are the best conditions for growth rates. The cellular morphology from the bacteria grown under 0,25 and 50 gr/L of NaCl varied lightly in shape, however all presented flagella. Then, under this same conditions motility assays and biofilm production were carried out, for motility we found that this significantly increases in 25 gr/L and it was completely inhibited at 50 gr/L, on the other hand, biofilm formation increases proportionally with the NaCl concentration. These results are correlative with the gene expression of *fliG* and *fliS* (Motility), *opuBA, putP, glnA, proC, gltA* and *gbsA* (transport and compatible solutes); *ywqC, bdlA, luxS* y *pgaC* (biofilm); therefore, we conclude that this strain effectively modifies the gene expression and physiology in a differential manner facing these diverse concentrations of NaCl for its own survival.

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CLP3-20

Water content and gypsum textural properties as determinants of endolithic colonization patterns in Salar de Pajonales, Northern Chile

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Salar Pajonales (SP), an Andean salt flat located at 25°10'S/68°49'W and 3,517 masl., presents high UV radiation, broad diurnal and annual temperature variations, low relative humidity and it is associated to volcanic activity. The water geochemistry, and the mineralogy and the water content of sediments and organo-sedimentary structures (OSS) of gypsum were studied, the microbial diversity was also assessed by microscopic techniques, 16S rRNA sequencing, and diversity indexes, and compared between samples in order to obtain colonization patterns. Water sample analyses show a concentration process probably due to evaporation. The water content of sediments and OSS decreases from west to east, determined by the hydrology and geomorphology of the system. *In Situ* Vis-NIR and Raman spectroscopic analyses of SP samples evidenced the presence of clay minerals and gypsum with pigments like chlorophyll, carotenoids, and scytonemin in colored microbial layers. Additionally in the lab, we detected the occurrence of coccoid cyanobacterial cells in OSS compatible with *Chroococcidiopsidales*, which confirms endolithic colonization along fossil diatoms. Besides, confocal laser scanning microscope (CLSM) λ-scan images and emission spectra evidenced that the mentioned cyanobacterial cells show fluorescence emitted by chlorophyll-*a* and phycobiliproteins higher than non-specific autofluorescence induced by degradation products of photosynthetic pigments. 16S rRNA gene clone library's results evidenced that the microbial community is dominated by Cyanobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, Planctomycetes, Actinobacteria, Euyarchaeota, Thaumarchaeota phyla. Additionally, Cyanobacteria/Bacteroidetes and Proteobacteria abundances are directly and indirectly related, respectively, to the water content in sediment and OSS samples. Diversity indexes indicate lower diversity, homogeneity, and richness of species in drier areas. In particular, we detected a low diversity of photosynthetic cells in the gypsum microhabitat where most sequences recovered greatly resemble those of *Microcystis*, Uncultured *Cyanobacterium*, and *Chroococcidiopsis* genus. These results contribute to the understanding of the microbial diversity on this system and highlight restrictive conditions of OSS for microbial colonization. Our findings provide direction for future studies where the combination of extreme climate variability and global climate decline (e.g. aridification, extreme radiation, high-temperature variability) would have led to habitat fragmentation and most likely, to the hyper-specialization of microorganisms in response to very specific habitat conditions.

Proyecto Donación Minera Escondida Limitada.

CLP3-21

Genomic data mining of the marine *Streptomyces* sp. H-KF8 and functional response to abiotic stressors

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The marine ecosystem is highly diverse, with extreme abiotic selective pressures and immense biological diversity. In spite of all the isolation studies associated to marine actinobacteria, such as *Streptomyces*, relatively little is known about the molecular mechanisms behind bacterial adaptation to marine environments. It seems that marine streptomycetes have adapted through the development of specific biological traits. Genome mining has become a powerful tool to unveil the potential of *Streptomyces* species. In this study, we aimed to conduct a combined genomic, metabolic and physiological analysis of the marine *Streptomyces* sp. H-KF8 bacterium. The genetic and functional response to abiotic stressors such as oxidative stress, heavy metals and antibiotics, which may play an important role in the evolution of secondary metabolism genes, was evaluated. A genomic search in *Streptomyces* sp. H-KF8 unveiled the presence of a wide variety of genetic determinants related to heavy metal resistance (49 genes), oxidative stress (69 genes) and antibiotic resistance (97 genes). This study revealed that the marine-derived *Streptomyces* sp. H-KF8 bacterium has the capability to tolerate a diverse set of heavy metals such as copper, cobalt, mercury, chromate and nickel; as well as the highly toxic tellurite, a feature first time described for *Streptomyces*. In addition, *Streptomyces* sp. H-KF8 possesses a major resistance towards oxidative stress, in comparison to the soil reference strain *Streptomyces violaceoruber* A3(2). Moreover, *Streptomyces* sp. H-KF8 showed resistance to 88% of the antibiotics tested, indicating overall, a strong response to several abiotic stressors. The combination of these biological traits confirms the metabolic versatility of *Streptomyces* sp. H-KF8, a genetically well-prepared microorganism with the ability to confront the dynamics of the fjord-unique marine environment.

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CLP3-22

Evaluation of the effect of sucralose on the intestinal microbiome in adults using next-generation sequencing

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Non-nutritive sweeteners or artificial sweeteners (NNS) are nutritional supplements consumed by millions of people around the world. They are aimed to prevent weight gain and diabetes, without an increase in caloric intake (Zheng *et al.*, 2015; Pepino, 2015). While they are considered as safe, there is a growing controversy in terms of its potential to promote metabolic alterations. Recently it has been shown that consumption NNS could induce intolerance to glucose in mice and humans, due to the modification of the intestinal microbiome (Suez *et al*., 2014; Suez *et al*., 2015). Considering the importance of noncommunicable diseases such as obesity and diabetes type 2 in our country, we determined the effect of sucralose on the metabolism of glucose in healthy volunteers and evaluated the effect of sucralose on the intestinal microbiome. A clinical double-blind study with 34 healthy men, who did not regularly consumed NNS, was performed. Subjects were divided into a control group and a treatment group. The control group received a placebo, while the treatment group received a dose of 300 mg daily of sucralose, both for 7 days. At the beginning and at the end of the study we determined the levels of glucose and insulin, as well as the composition of the intestinal microbiome of the subjects through extraction of fecal DNA, followed by massive sequencing of the 16S rRNA gene and bioinformatic sequence analysis. The clinical study results did not reveal statistically significant differences in the groups in glucose or insulin levels after the study. Also, sequencing of 16S rRNA of the gut microbiome indicated that there were not significant changes in the composition of the microbiome before and after administration of 300 mg daily of sucralose for 7 days. This study suggests that sucralose at high dosage does not alter the metabolism or gut microbiome of healthy subjects in a short time term.

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CLP3-23

Phylogenetic analyses of Antarctic fungi related to the genera Geomyces, *Pseudogymnoascus* and *Pseudoeurotium*

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To date, few species from the fungal genera *Geomyces*, *Pseudogymnoascus* and *Pseudoeurotium* have been described. In Antarctica, fungi from these genera, especially *Pseudogymnoascus*, seem to be prevalent in most of the ecosystems studied so far. In our laboratory, we have a large collection of Antarctic fungi isolated from marine sponges that according to their morphological characteristics could belong to *Geomyces*, *Pseudogymnoascus* or *Pseudoeurotium*. To gain insight about the diversity of these Antarctic isolates, in this work we have performed the phylogenetic analysis of 57 of these strains using suitable molecular markers. These markers were amplified by PCR, sequenced, and the sequences obtained were concatenated and used to construct several phylogenetics trees. The analysis of these trees suggests that some of these isolates belong to the species *Pseudogymnoascus verrucosus*, whereas other ones are closely related to *Pseudoeurotium zonatum*. However, most of the isolates cannot be ascribed to a previously known species, suggesting that they are representative of non-described species. Some of these putative new species are lightly related to *Pseudogymnoascus roseus*, *Pseudogymnoascus destructans* and *Geomyces auratus*, but the vast majority does not show any relationship with previously described species. Our results suggest that Antarctica could contain more new species from these fungal genera than all those currently known.

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CLP3-24

Transcriptomic shift in metabolic related genes of *Stigonematal* cyanobacteria in response to the thermal gradient of Porcelana hot spring

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Cyanobacteria are bacterial organisms characterized by its capacity to obtain their energy from the sun, fix carbon dioxide to organic carbon compounds, and in some cases, fix N_2 to ammonium. However, it has been reported that some cyanobacteria can grow on sugar-supplemented media under conditions of photosynthesis inhibition, such as darkness or chemical inhibition, indicating that cyanobacteria may be mixotrophs rather than strictly photoautotrophic organisms. In the environment, Cyanobacteria are one of the most important primary producers, in particular when eukaryotes primary producers cannot grow like in thermal systems. In Porcelana hot spring, the Stigonematal cyanobacteria *Mastigocladus* is the main primary producer and nitrogen fixer at low temperatures in the thermal gradient (from 48ºC to 66ºC). Based on our own metatranscriptomic data, and consistently with previous studies, we found that most *Mastigocladus* genes were repressed by high temperature. However, our data indicates that *Mastigocladus* are still active at high temperatures and, as expected, stress response and protein metabolism associated genes were induced by temperature. Notable genes categories that were repressed by temperature include nitrogen fixation and photosynthesis, suggesting that temperature is limiting the autotrophic activity of *Mastigocladus* at transcriptional level in the system. Contrary, genes related to carbohydrate consumption, like sugar channels and glycolysis-associated genes as well as genes related to the amino acid metabolism were induced by high temperature, suggesting that *Mastigocladus* may change to a heterotrophic-like metabolism. In the laboratory, we grown *Mastigocladus* sp. strain CHP1 (isolated from Porcelana hot spring) at high temperatures in BG11 $_{NO3}$ medium supplemented with glucose, finding that supplemented cultures were more thermostable that those without glucose supplementation, which corroborate that *Mastigocladus* members can use external organic carbon sources to survive under high temperature conditions. Discovering how temperature affects the metabolic state of Mastigocladus could help us to better understand the contribution of these relevant organisms in the carbon cycle in thermal systems.

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CLP3-25

The complexity of the N-glycosylation at the position 144 on the hemagglutinin of the Influenza A Virus determines the avidity for its receptor and the evasion of the host immune response

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During evolutionary adaptation of Influenza A Virus (IAV), antigenic drift is a key process that integrates mutations that eventually become fixed in the surface of the Hemagglutinin (HA) glycoprotein. This is the immunodominant protein of the virus that recognizes the sialic acid receptors in the host cells. One strategy used by IAVs to evade pre-existing immunity (antibodies) is the addition of N-glycosylation sites, particularly in the globular head of HA. We have shown that a glycosylation at the position 144 of the 2009 H1N1 HA can induce a polyclonal response capable of neutralizing other glycosylated H1N1 variants. This glycosylation was also very effective at shielding the antigenic site Sa of HA. We hypothesized that the complexity of glycosylation 144 modulates the antigenic properties of HA by redirecting the humoral immune response to other antigenic sites. To evaluate the characteristics of this glycan, we used variants of the A/Netherlands/602/09 H1N1 virus HA protein with glycosylations at sites 144, 142, 172, and 144-172, which were expressed and purified for N-glycan analysis by CGE-LIF (Capillary Gel Electrophoresis with Laser Induced Fluorescence). We also produced recombinant viruses harboring these HA glycosylation variants, in the presence or absence of the mannosidase inhibitor, kifunensine, which generated either high-mannose or complex glycosylations, respectively. Analyses of these HAs by Western Blot showed a higher molecular weight of the HAs containing the 144 glycosylation in both, the recombinant viruses and soluble proteins, indicating that this position allows the assembly of longer glycans compared to glycosylations in other nearby positions. Reducing the length of the glycans increased the avidity of HA for its receptor, suggesting that glycosylations near the receptor-binding site might have an impact on viral fitness. We performed immunizations in C57BL/6 mice to assess if the complexity of N-glycosylations on site 144 is sufficient to modulate the humoral response, or whether there is an intrinsic effect of this glycosylation that modulates the antigenic properties of HA. These results will provide new insights of the biological relevance of the N-glycosylation at site 144 in the IAV HA, contributing to future considerations in the development of more efficient vaccines.

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CLP3-26

Contribution of *mnt3* in sclerotia formation and adhesion in the ubiquitous plant pathogen *Botrytis cinerea*

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Botrytis cinerea is a phytopathogenic fungus that infects different plant species and is a cause of large crop losses in the local and world agricultural industry. The primary contact of the fungus with its host takes place at the cell surface. Therefore, the fungal cell wall plays an important role in fungal pathogenesis. The genome of *B. cinerea* contains over 100 putative glycoproteins that can be anchored to the cell wall through a glycosylphosphatidylinositol (GPI) moiety or can be linked directly to the β-glucans, such as the PIR1 protein. Glycoproteins are *N*- and *O*-glycosylated polypeptides; glycosylation occurs by a process that involves various enzymes, such as those encoded by the mnt gene families. This study seeks to establish the importance of the *N*- and *O*-glycosylation pathways for the cell wall integrity, adhesion and virulence of *B. cinerea*. Therefore, the aims are to determine the contribution of *MNT3* in the *N*- and/or *O*-glycosylation processes in *B. cinerea* and to elucidate their role in pathogenesis. To achieve this, we made the *mnt3* mutants by homologous recombination. Single spore isolates for each knockout was confirmed by RT-PCR. The cell wall integrity in the mutant was analyzed by measuring the sensitivity to different antifungal drugs, including Calcofluor White (CW) (alters the assembly of chitin fibrils in the cell wall), Congo Red (CR) (alters the assembly of microfibrils of ß-1,3-glucan), SDS (detergent which perturbs the integrity of the cell membrane). Virulence assays was tests in tomato fruits and leaves. Both, tomato leaves and fruits developed typical gray mold symptoms after 4 days of inoculation. Preliminary evidence indicates that *MNT3* affects sclerotia formation and adhesion in *Botrytis*, but it is not involved in cell wall integrity and virulence. Further studies are necessary to determine if *MNT3* gene is involved in N and O Glycosylation.

ICM MINECON MNFISB

CLP3-27

Isoflavonoids as modulators of reactivation of HIV-1 latency

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One of the major challenges for the cure of HIV-1 infection is the persistence of proviral genomes in CD4 + T lymphocytes. Specific approaches are being sought to reverse latency and use immunological mechanisms to eradicate persistent infection. HIV-1 Latency Reversing Agents (LRAs) are small molecules capable of modulating the pathways that control HIV-1 latency and are the first target molecules in this effort. Isoflavonoids represent a class of potentially useful components to counteract HIV-1 infection. These compounds are found in vascularized plants and possess a variety of cellular and biochemical effects in animals. Isoflavonoids have been reported to affect viral replication and translation mechanisms, stimulate induction of both cytokine transcription and secretion factors, and their ability to activate the latent virus in JLR2 and ACH-2 cell lines has also been described. Their efficacy against viral infection *in vitro* is dependent on the dose and combination of the Isoflavonoids used. Based on these data, we evaluated the effect of 14 synthetic isoflavonoids on Jurkat-based cell line containing a full-length integrated HIV-1 genome that expresses GFP upon activation. Cells were treated with micromolar concentrations of isoflavonoids (20 to 200 µM) and latency activation was assessed by i) GFP expression by flow cytometry and ii) HIV-1 RNA quantification by qRT-PCR. Results showed that four isoflavonoids generate an activation of 16% at 20 µM compared with the control group. These results suggest that isoflavonoids modulate viral replication in latently infected cells, and these data may provide some guidance for the evaluation and application of new isoflavonoids as latency reactivators.

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CLP3-28

Genomic and proteomic evidence of a type IV pili-like structure in the fish pathogen *Piscirickettsia salmonis*

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Piscirickettsia salmonis is an intracellular γ-proteobacteria of the order *thiotrichales*, and the etiological agent of salmonid rickettsial septicaemia (SRS), which causes massive economic losses to the Chilean salmon industry. An important virulence attribute in pathogenic bacteria is their ability to adhere to host cell surfaces. To this purpose bacteria have developed many strategies, the use of pili being one of the most prevalent. Pili are appendages that extend from the bacterial membrane, are usually formed by multiple polymers of a single protein, their biogenesis and function depending on a complex machinery. The type IV pilus (Tfp) is the most widespread among bacterial lineages, comprising a variable number of components among species, some of which have been predicted on *P. salmonis* genomes, but no studies have been conducted on whether *P. salmonis* possesses a Tfp. Therefore, the goal of the present study was to identify Tfp components in *P. salmonis* strain LF-89, evaluate their transcript expression and analyze their putative proteins using bioinformatic and proteomic approaches. Results show that *P. salmonis* Tfp genes are organized in two main clusters (*pilABCD* and *pilMNOPQ*) with conserved synteny among related bacteria and are actively transcribed during *in vitro* infection. The *pilA* gene, encoding the main pilus subunit, shows a peak in expression 4 hours after infection, while the secretin-encoding *pilQ* shows an upregulation 4 days post-infection. Notably, the *pilB* and *pilD* genes, which encode an ATPase and a peptidase respectively, are strongly up-regulated 16 hours post-infection. Putative protein sequences were analyzed with various tools (SignalP, TMPred, PSORTb, InterProScan) which allowed to infer subcellular localization and function. Analysis of the putative PilA protein showed a conserved N-terminal domain and sequence motifs which are critical to Tfp biosynthesis. A structural model displays a similar architecture to PilA from related γ-proteobacteria. Also, PilA was identified in the proteome of *P. salmonis* LF-89 by MudPit analysis (8 unique peptides with 25.18% coverage) and pilus-like filamentous structures were observed in the surface of *P. salmonis* by TEM. Taken together, these results suggest the presence of a Tfp in this fish pathogen.

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CLP3-29

The OxyR transcriptional regulator protects *Burkholderia xenovorans* LB400 against oxidative stress and contributes to the metabolism of aromatic compounds

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Burkholderia xenovorans LB400 is a bacterium that degrades aerobically a wide range of aromatic compounds, whose metabolism may generate a stress condition. The bacterial response to oxidative stress in *B. xenovorans* has not been described. The regulon OxyR regulates the oxidative stress in *E. coli*, activating the expression of diverse antioxidant enzymes. The goal of this study was to characterize the oxidative stress response in *B. xenovorans* strain LB400 to oxidizing agents and the metabolism of aromatic compounds. Bioinformatic analysis showed the presence of diverse genes involved in the oxidative stress in the genome of the strain LB400 including the *oxyR* gene (BxeA3087) that encodes the transcriptional regulator OxyR. The *oxyR* gene was located upstream of the *recG* gene. The *katG* and *dpsA* genes were located downstream of the *oxyR* gene, which belong to the OxyR regulon in *E. coli*, and whose gene organization is similar to that observed in Betaproteobacteria. The *oxyR* gene was overexpressed using the low-copy vector pIZ1016 (a pBBRMCS derivative) in *B. xenovorans*. The formation of reactive oxygen species (ROS) was monitored during exposure to H_2O_2 and paraquat (20 mM) using the HPF probe for hydroxyl radical. Overexpression of the *oxyR* gene in strain LB400 decreased ROS formation and increased the viability (UFC/ml) of *B. xenovorans* during exposure to paraquat (20 mM). Cells overexpressing *oxyR* gene attained higher turbidity on 3- and 4-hydroxyphenylacetate, suggesting that OxyR improves oxidative stress response during metabolism of aromatic compounds. These results indicates that the *oxyR* gene protects *B. xenovorans* against oxidative stress generated by paraquat and during degradation of aromatic compounds.

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CLP3-30

Molecular systematics of the genus *Acidithiobacillus*: insights into the phylogenetic structure and diversification of the taxon

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The acidithiobacilli are sulfur-oxidizing acidophilic bacteria that thrive in both natural and anthropogenic low pH environments. They contribute to processes that lead to the generation of acid rock drainage in several different geoclimatic contexts, and their properties have long been harnessed for the biotechnological processing of minerals. Presently, the genus is composed of seven validated species, described between 1922 and 2015: *Acidithiobacillus thiooxidans*, *A. ferrooxidans, A. albertensis*, *A. caldus*, *A. ferrivorans*, *A. ferridurans* and *A. ferriphilus*. However, a large number of *Acidithiobacillus* strains and sequence clones have been obtained from a variety of ecological niches over the years, and many isolates remain unclassified and several conflicting specific assignments muddle the picture from an evolutionary standpoint. In this work, we revise the phylogenetic relationships within this species complex and determine the phylogenetic species boundaries of sequences affiliated to the *Acidithiobacillus* spp. collected from public and private databases using three different typing approaches with varying degrees of resolution: 16S rRNA gene-based ribotyping, oligotyping, and multi-locus sequencing analysis (MLSA). Results obtained indicate that there is still considerable unexplored diversity within this genus. At least six new lineages or phylotypes, supported by the different methods used herein, were identified. Also, varying levels of intra-linage diversity were detected. Draft genomes of cultured representatives of the new lineages and divergent branches were sequenced to further explore the genomic landscape of the taxon. The revised phylogenetic structure for the *Acidithiobacillus* genus presented herein paves the way for future population genomics and evolutionary studies in this important acidophile model.

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CLP3-31

Genome sequence analysis of *Pseudomonas* sp. K2I15: an Antarctic bacterium producing bacteriocin-like polypeptides

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Background. The genus *Pseudomonas* is one of the most diverse and ecologically significant bacterial groups on the planet. The members of this genus have been isolated from different environments, such as terrestrial, aquatic, marine and associated with plants and animals. This universal distribution suggests a wide range of physiological and genetic adaptability, allowing a remarkable ability to produce, for example, antimicrobial molecules. The objective of this study was to identify a cluster of genes that encoded a putative bacteriocin in the genome of the *Pseudomonas* sp. strain K2I15, isolated from the rhizosphere of the *Deschampsia antarctica* Desv. plant. **Methods.** From a culture of *Pseudomonas* sp. in LB broth at 18 ° C, genomic DNA was isolated by QIAamp DNA minikit (Quiagen). A sequencing library was prepared using the Illumina HiSeq (MicrobesNG, UK) platform. 17,514,936 paired ends are used to perform the reassembly in Geneious. Results were 140 contigs with an average coverage of 97X (N50, 94,045 bp). The annotation was performed using PGAAP (NCBI). Additionally, the genome was analyzed using the RAST server. Antibiotic resistance genes acquired were identified using ResFinder 2.1, virulence factors by VirulenceFinder 1.2 and CRISPR by CRISPRfinder. The search for possible bacteriocins is done through AntiSmash and its physic-chemical characterization using ProtParam. **Results.** The final assembly had a total length of 6,645,031 bp, and a GC content of 60.4%. Genome annotation resulted in 5,981 coding sequences (CDS), 59 tRNAs, 104 pseudo genes, and 7 rRNAs. No virulence genes or CRISPR arrays were identified. Niche adaptation factors with functions such as copper, arsenic and zinc tolerance were recognized. Cold shock CspA-I family of proteins, production of siderophore (pyoverdine, acromobactin) and the production of colicin V and auxin genes were detected in *Pseudomonas* sp. genome. Antibiotic resistance genes were found for fluoroquinols, streptocry- mic, penicillin and fosfomycin. Two genetic cluster were identified that encode possible bacteriocins with physicochemical characteristics of a bacteriocin microcin type. **Conclusion.** This genome sequence allowed to identify two genetic clusters that codify possible bacteriocins with antimicrobial activity of the microcin type.

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CLP3-32

Macrophage disruption by *Piscirickettsia salmonis* and use of immune complex to control of bacterium infection

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Background. The most recurrent bacterial diseases in Chile is Piscirickettsiosis. The aetiological agent is *Piscirickettsia salmonis* who caused about 74.6 % of mortality by infectious of salmo salar cause in 2016 (SERNAPESCA). This intracellular facultative Gram-negative bacterium is able to survive, replicate and propagate on macrophages. The mechanisms display by *P. salmonis* to survive is not fully understood. Our aim is to explore this process describing the bacterial survival, replication in the cell and immune response of primary culture of infected macrophages. The results were used to develop on a new immunization strategy based on an immune complex **Methods.** Primary culture of *S. salar* macrophages was infected with *P. salmonis-FITC,* the intracellular localization and bacterial replication was evaluated by confocal microscopy and qPCR of 16S rDNA of *P. salmonis* respectively during six days post infection. In addition, we determinate if *P. salmonis* was able to modify the macrophages expression-transcript of TNF-α, TGF-β, IL-1β, IL-10, IL-18, IL-12, MHC- I and MHC-II by qRT-PCR. Finally we develop immune-complexes (IC) against *P. salmonis* (confidential design), and then we was evaluated the cytopathic effect and replication of *P. salmonis* at 72h and 120h post-infection in macrophages treated with immune-complexes. **Results.** We observed a slight increase on expression of transcripts of pro-inflammatory cytokines accompanied with an increase of transcripts of anti-inflammatory cytokines against *P. salmonis.* The bacterial load reaches an intracellular peak after 72h post-infection, suggesting that is not degraded by macrophages. Surprisingly, the addition of IC to the macrophages infected with *P. salmonis* decreased the bacterial load accompanied by a reduction of cytopathic effect in macrophages. **Conclusion.** Our results suggest that the macrophages infected with *P. salmonis* induce a poor inflammatory response that could facilitate the intracellular residence of this pathogen, and the use of immune complexes favor the reduction of bacterial load, suggesting a re-activation of infected macrophages.

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CLP3-33

Contribution of hRSV to the susceptibility to mycobacterial infection in a mouse model

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The human respiratory syncytial virus (hRSV) is the principal cause of childhood hospitalization due to severe lower respiratory tract infection. HRSV infection alters the function of immune cells that are primordial in the acquisition of immunity to pathogens, such as dendritic cells (DCs) and T cells.The effect over the lung epithelial cells induced by the immune response triggered by hRSV infection and the predisposition and susceptibility to a subsequent bacterial infection remains unknown. Here we evaluated whether hRSV may generate a susceptible environment to subsequent infection using *Mycobacterium bovis* as a model the infection, which belongs to the *Mycobacterim tuberculosis* complex. For this purpose, C57BL/6 mice were infected with hRSV and ten days post-infection were challenged with *M. bovis*. Eleven days-post infection mice were euthanized and the lung tissue was evaluated by flow cytometry to determine immune cell populations, RT-PCR to establish the expression of various genes and bacterial culture to determine the loads of *M. bovis*. Our data suggest that a previous exposure to hRSV significantly increased the susceptibility to infection by *M. bovis* and enhanced lung pathology. Importantly, increased pathology associated with enhanced bacterial loads, as observed by increased copies of *M. bovis* 16S rRNA gene, suggesting that clearance of the infecting bacilli was impaired due to the previous exposure to hRSV. In addition, histopathological analyses of lung sections of infected mice showed red-orange histiocytes, which are structures that correlate with a dormant-like phenotype of *M. bovis*. Further, these events associated to an increase expression for the heme oxygenase -1 gene, which contributes to the histiocytes generation. Furthermore, our results suggest that this poor anti-mycobacterial response is not due to a decrease in the T cell response to mycobacterial antigens, but due to alterations in the functionality of APCs in the lungs of hRSV-infected mice.

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CLP3-34

Vat and Sat, two SPATEs contributing to the adherent-invasive *Escherichia coli* (AIEC) pathogenic phenotype

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Adherent-invasive *Escherichia coli* (AIEC) strains, have been linked to the pathogenesis of Crohn disease (CD). AIEC strains are genetically diverse and are characterized by their ability to: i) adhere to epithelial cells, ii) invade epithelial cells, and iii) survive within macrophages. However, the pathogenicity mechanism of AIEC is still not well resolved. One of the most studied virulence factors in AIEC strains is *fimH*. This gene encodes an adhesin located at the tip of the Type 1 pili. In addition, recent studies have suggested that serine proteases autotransporters of the *Enterobacteriaceae* family (SPATEs) could be involved in promoting intestinal colonization of AIEC. SPATEs are multifunctional virulence factors that can have intracellular or extracellular protein substrates in host tissues. The goal of this study was to investigate the contribution of vacuolating autotransporter toxin (Vat) and secreted autotransporter toxin (Sat) SPATEs in the AIEC phenotype. The detection of genes encoding Sat and Vat was performed by PCR and proteins were detected by Western blotting. In order to evaluate their function, *vat* and *sat* genes were inactivated in AIEC 18C02 and 6C03 strains, respectively, by allelic replacement. Adhesion assays were performed using Caco-2 cells and the survival and replication into macrophages were evaluated in Murine RAW 264.7. We found that seventeen of forty-seven AIEC strains analyzed were positive for *sat* (36.2%, 17/47) and ten of forty-seven were positive for *vat*. Only the Vat mutant (18C02Δ*vat*) exhibited a significantly lower level of adhesion ($p<0.05$) to Caco-2 cells compared to the wild-type. In contrast, there were no significant differences between the level of adhesion of 6C03Δ*sat* and the wild-type strain ($p>0.05$). Survival of the 18C02Δ*vat* strain in macrophages decreased significantly after three hours of infection, compared to wild type strain but, at 24 h the difference was not observed. In the other hand, survival of 6C03Δ*sat* was not significantly affected at any time. However, expression of *vat* or *sat* conferred survival capacity to the non-pathogenic *E. coli* HB101 strain. In conclusion, Vat contributes to the AIEC phenotype by mediating adherence and survival within macrophages. In contrast, Sat would contribute only to the survival within macrophages.

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CLP3-35

Integrity and viability of *Piscirickettsia salmonis* when infects Atlantic salmon macrophages

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Background. *Piscirickettsia salmonis* is the most important health problem that facing Chilean aquaculture industry. In the last years has been suggested that *P. salmonis* infects and replicates within macrophages avoiding its lysosomal degradation. However, the host-pathogen interaction involved in survival and replication are poorly understood. in this work, we have proposed to evaluate both bacterium localization respect to lysosome; as also integrity, survival and replication of *P. salmonis* when infects Atlantic salmon macrophages. **Methods.** To identify lysosomes we incubated macrophages with ferritin previous to infect with *P. salmonis*. Ferritin accumulation in electron-dense organelles is an indicator of the lysosomes localization. To evaluate localization and also the integrity of *P. salmonis,* we developed a time-course infection in ferritin-loaded macrophages between 3 and 48 hours. The host-pathogen interaction was analyzed by transmission electron microscopy (TEM). Survival and replication analysis were developed in Atlantic salmon macrophages infected with *P. salmonis* during 6, 24, 72 and 120 hours by a Gentamicin protection assay followed to qPCR and units forming colonies (UFC) in CHAB agar quantification. The *P. salmonis* quantification was done both for intracellular bacterium as also present in the infected supernatant. **Results.** In TEM images we observed that *P. salmonis* appeared intact within ferritin free-vacuoles even 48h post-infection, they were not detected in ferritin loaded-vacuoles. By the contrary, bacterium fixed with PFA incubated with macrophages at the same times appeared with its membrane degraded and within the electron-dense vacuoles. Gentamicin protection assay followed by quantification of *P. salmonis* showed that the intracellular bacterium reached a maximum after 72h post-infection, coinciding with the first detection of *P. salmonis* in supernatant of infected macrophages, where reached a maximum number at 120 hours post-infection. **Conclusion.** Finally, our results suggest that *P. salmonis* does not reach lysosome when infects Atlantic salmon macrophages and resides within vacuoles where in addition to survive it seems to be replicating.

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CLP3-36

Impact of iron availability and medium acidity on lipid A structure in *Helicobacter pylori*

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Helicobacter pylori is a Gram-negative pathogen that colonizes the gastric epithelium of humans, causing gastritis, peptic ulcers and gastric cancer. One of the most remarkable characteristics of this bacterium is its ability to generate persistent infections that may last decades, which may explain its widespread worldwide dissemination. One of the factors that promotes the persistence of this pathogen in the human stomach is the unusual structure of its lipopolysaccharide (LPS). This complex molecule presents modifications in the O antigen (OAg) which contribute to molecular mimicry. Moreover, its lipid A presents a highly modified structure, granting this molecule a reduced immunoreactivity. Despite these extensively described features of *H. pylori* LPS, the environmental cues that regulate these modifications have not been reported. In this study, we evaluated the effect of pH and iron availability, both being relevant conditions for *H. pylori* pathogenicity, on lipid A structure. For this purpose, LPS was purified from bacteria grown under different conditions and analyzed by negative-ion mass spectrometry. First, bacteria were cultured in rich medium adjusted to pH 7 or pH 5. The lipid A mass distribution profile of bacteria grown at pH 7 presented several species grouped as duplets and triplets. In the case of bacteria grown at pH 5, an increment in the relative abundance of the species with the highest mass was observed for each group, with a concomitant decrease in the relative abundance of their lower mass counterparts. Next, bacteria were cultured in standard rich medium and rich medium supplemented with iron (Fe^{+2}, 1 mM). The mass profile revealed that under both conditions, bacteria produced the same species of lipid A observed before, also grouped as duplets and triplets. However, an increment in the relative abundance of the species with the lowest mass of each group was observed when bacteria were grown in medium supplemented with iron. Together, these data showed that environmental conditions similar to those faced by *H. pylori* in the human stomach modulate the structure of its LPS, which consequently could affect the virulence of this pathogen.

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CLP3-37

Trypanosoma cruzi trypomastigotes and amastigotes present in the meat of acutely infected mice induces the mammalian infection by oral route

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Trypanosoma cruzi infection is still prevalent in Latin America, where despite control strategies, active transmission still occurs. In this context, the high prevalence of human infection in some areas where *T. cruzi* triatomine infection is low, has allowed the hypothesis that the consumption of raw or undercooked meat from *T. cruzi* infected animals could be responsible for transmitting the infection. However, this hypothesis has not been demonstrated and there is currently no evidence that the consumption of meat from infected animals could transmits the infection. Therefore, the aims of this works was "To assess experimentally the role of infected animals meat consumption in the oral transmission of *Trypanosoma cruzi* infection". Groups of five female BALBC mice, were fed with muscles obtained from mice infected with *T. cruzi* in both acute and chronic phase of the infection. The infection was monitored by parasitaemia curves. In the same way, as these stages correspond to those that potentially would be found in meats of *T. cruzi*, infected animals, the infective capacity of trypomastigotes and amastigotes from the *T. cruzi* clone H510 C8C3, was evaluated, infecting groups of five mice BALBC Females, who were infected by mouth using a naso-gastric tube. The infection was monitored by study of the parasitaemia curve. Finally, different inoculums of trypomastigotes and amastigotes were used to determine their infective capacity and the murine infection by *T. cruzi* was monitored by curve of parasitaemia studies. In all cases, the 80 to 100 percent of the meat-fed animals from mice in acute phase of *T. cruzi* infection, or infected by mouth via naso-gastric tube with tripomastigotes or amastigotes, were infected. These animals, developed high parasitemias and 50% of them died around the day 40 post infection. It is concluded that the consumption of meat from infected mice in acute phase of the infection, allows the oral infection by *T. cruzi*. Similarly, both trypomastigotes and amastigotes were able to induce mice-infections when administered orally.

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CLP3-38

Effect of Hemoxigenase 1 induction in the severity of *Salmonella enterica* serovar Typhimurium infection in mice

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Salmonella enterica is the leading cause of infectious gastroenteritis and systemic diseases in humans. The main virulence trait of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is the ability to avoid the immune response of the host. The specific factors that participate in the ability of *S.* Typhimurium to avoid immune response are not totally elucidated. One of the immunomodulatory molecules expressed by the host that has a role in bacterial clearance is Heme oxygenase 1 (HO-1). HO-1 is an enzyme that catalyzes the heme degradation reaction that releases Fe^{3+}, biliverdin and carbon monoxide (CO). The role of HO-1 production during *S.* Typhimurium infection is not clear and previous studies show contradictory results. In this study we proposed to induce HO-1 expression in mice prior to infection with *S.* Typhimurium. To achieve this, we treated C57BL/6 mice with Cobalt Protoporphyrin (CoPP), an inducer of HO-1 expression, and after 24 h mice were infected with *S.* Typhimurium. Weight change, clinical score and survival were measured daily. After 5 days of infection we determined bacterial loads in spleen, liver, feces, blood and mesenteric lymph nodes. Our results show that infected and CoPP-treated mice showed a tendency to survive more than infected-untreated mice, and to decrease bacterial load at 5 days post- infection in spleen, liver and feces. Interestingly, bacterial load in blood of infected and CoPP-treated mice was higher than in infected and untreated mice. Our results suggest that HO-1 plays an important role in *S.* Typhimurium clearance and dissemination to deeper tissues in mice.

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CLP3-39

Evaluation of antimicrobial resistance and cell invasion capacity of *Staphylococcus aureus* strains, according to their persistence or transience in the bovine mammary gland

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Staphylococcus aureus is one of the most prevalent agents of bovine mastitis in the world. Its virulence is based predominantly on its ability to invade host epithelial and phagocytic cells, allowing it to evade the host´s immune system and antimicrobial treatments. *S. aureus* is able to remain for long periods of time in the bovine mammary gland, causing chronic mastitis refractory to antibiotic treatment. For this study, previously, in monthly samples for a period of one year, the presence of *S. aureus* in milk samples from cows with mastitis was identified. Isolates of *S. aureus* capable of remaining in the bovine mammary gland were persistently ("persistent" strains), and others that remained transiently ("transient" strain) in the mammary gland were identified. The aim of this study was to determine if there are differences between persistent strains and transient strains respect to their *in vitro* capacity of cellular invasion and antimicrobial resistance. A total of 36 isolates were preselected and characterized by PFGE, allowing the identification of "persistent" *S. aureus* strains and "transient" *S. aureus* strains. These strains were characterized in terms of: 1) antimicrobial resistance by disc diffusion method and 2) *in vitro* capacity of invasion of human mammary epithelial cell (MCF-7). All strains were sensitive to the 13 antimicrobials tested (methicillin, ampicillin, oxacillin, amoxicillin, gentamicin, amikacin, streptomycin, ciprofloxacin, enrofloxacin, vancomycin, erythromycin, clindamycin and lincomycin). Regarding invasion assays, "persistent" *S. aureus* strains had a greater ability to invade MCF-7 cells than "transient" *S. aureus* strains ($p < 0.05$). Finally, there are differences between persistent strains and transient strains respect to their *in vitro* cell invasion ability, but not respect to antimicrobial resistance. In future assays, the selected strains will be evaluated in terms of *in vitro* capacity of biofilm formation to determine differences in the ability of strains to persist in the mammary gland.

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CLP3-40

Dysbiosis induced by bacitracin/neomicin correlated with alterations on innate immune response parameters in *Salmo salar*

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In mammals and fishes, the role of the microbiota on host physiology has been studied through the preferential use of gnotobiotic animals or through the use of nonabsorbable antibiotics such as bacitracin/neomycin. In *Salmo salar* the effect of the microbiota on fish physiology is unknown, gnotobiotic specimens are lacking and studies of the effect of bacitracin and neomycin are not available. To study the effect of the microbiota on salmonids physiology, the hypothesized was: "In *Salmo salar*, the use of antibiotics neomycin/bacitracin causes changes in the composition of the gastrointestinal microbiota (dysbiosis), decreasing both the diversity of microorganisms present in the leukocyte populations found in the immune organs ". Methods Dysbiosis was induced by administration of neomycin/bacitracin to pre-smolt specimens for two weeks. The total bacterial load in feces and intestine was assessed by qPCR, quantifying of copies of the 16S ribosomal gene (16S rDNA). Community richness was assessed by amplification of a V3 variable region of the 16S rDNA gene, and gradient denaturing gels analysis. The effect on the immune system was evaluated by a) quantification the leukocyte populations in immunological organs by flow cytometry; and b) quantification the expression of cytokines such as IL1β, TNFα and TGFβ by RT-qPCR. The results show that antibiotic administration leads to a decrease in bacterial load, reduces the richness of microbial communities of feces and intestine, reduces leukocyte populations in the head kidney, and increases the expression of IL1β and TNFα. These effects did not reverse after antibiotic removal. Conclusions The administration of antibiotics neomycin/bacitracin causes dysbiosis in *Salmo salar* and generates an inflammatory state with reduction of the cellular immune response. The results suggest that the fishs normal microbiota regulates its immune response, stimulating lymphocyte populations, and an anti-inflammatory state.

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CLP3-41

The lost of genes involved in sensing alternative electrons aceptors modulates the pathogenicity of *Salmonella enterica* serovar Typhi

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Salmonella enterica is a pathogen causing disease in a wide range of hosts. The serovars Typhi and Typhimurium, infecting humans and a wide range of host respectively, present 97.6 to 99.5% of identity between their conserved sequences. We have hypothesized that host variability between both serovars could be due to mutations and formation of pseudogenes. In anaerobiosis, *Salmonella* requires alternative acceptors of electrons to complete efficient infection in the intestine. Strains of *S*. Typhi have lost functionality of genes involved in these pathways, such as *ttrRS* and *tsr*, whose products sense tetrathionate and nitrate respectively, both alternative electron acceptors. In contrast, *S*. Typhimurium presents these pathways intact, allowing efficient colonization of the intestine and promoting inflammatory processes. With the objective of determining the loss of these genes in *S.* Typhi and its impact on pathogenesis, we measured expression, mutated and heterologously expressed confirmed pseudogenes in *S*. Typhi. Expression of above mentioned genes was measured in *S*. Typhi and *S.* Typhimurium and in *tsr*, *ttrR* and *ttrS* mutants. Mutation of *tsr* gene in *S*. Typhimurium reduces the expression of *ttrR* and *ttrS*. In contrast, deletion of *tsr* in *S.* Typhi has no impact on expression of *ttrR* and *ttrS,* supporting the idea that *tsr* is a pseudogene in *S.* Typhi but not in *S*. Typhimurium.The *tsr* gene was restored in *S.* Typhi with the functional *tsr* gene of *S.* Typhimurium. This strain was used to determine adhesion, invasion and proliferation in cultured cells, showing a decrease in invasion and proliferation of *S*. Typhi tsr_{STM} heterologous strain compared with WT.To observe the differences *in vivo*, intraperitoneal infections of BALB/c mice were performed with mutants in *tsr*, *ttrR* and *ttrS* genes and *S*. Typhimurium WT parental strain. Mutants in *ttrR* and *ttrS*, showed a defect in the infective process compared with the WT strain, in contrast the *tsr* mutant, presented an increased proliferation within its host, compared to WT. On the light of our results we conclude that *tsr* could be disadvantageous for *Salmonella* to complete an intracellular and systemic infection, supporting the fact that is a pseudogene in *S*. Typhi.

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CLP3-42

Danish Recombinant BCG strain promotes a protective humoral immune response against the Human respiratory syncytial virus (HRSV) and Human Metapneumovirus (HMPV)

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Human Respiratory Syncytial Virus (hRSV) and Human Metapneumovirus (hMPV) are two of the leading etiology agents of acute lower respiratory tract infections (ALRTIs) worldwide affecting young infants, elderly and immunocompromised patients. Currently, there are no licensed vaccines against both viruses. We have previously described that immunization with recombinant Mycobacterium bovis Bacillus Calmette-Guérin (BCG) strains expressing the hRSV Nucleoprotein (rBCG-N) and hMPV Phosphoprotein (rBCG-P) are able to stimulate the cellular immune response triggering an effective protective role. Here we show that both vaccines promote protective humoral immunity, characterized by the production of antibodies specific against both antigens, as well as for other viral proteins. Also, we detected a consistent isotype switching from IgG1 to IgG2a, which correlated with an increased viral clearance, virus neutralizing capacities *in vitro* and protection in naïve mice from the pathology. These results support the notion that the use of recombinant BCG strains could be considered as potential vaccination approach against respiratory viruses, such as hRSV and hMPV promoting an efficient cellular and humoral immune response.

CONICYT, FONDECYT, INSTITUTO MILENIO DE INMUNOLOGÍA E INMUNOTERAPIA

CLP3-43

Evaluation of the antimicrobial cellular activity in SHK-1 cell line against *Piscirickettsia salmonis*

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Background. *Piscirickettsia salmonis* is an aggressive intracellular fish pathogen that causes Salmonid Rickettsial Syndrome (SRS) in salmonids. This syndrome generates large losses in Chilean salmon farming, threatening the sustainability of salmon production in our country. Currently, the control and prophylaxis strategies against *P. salmonis* has been based on the use of antibiotics and vaccines that have not had the expected success against infection, which makes it urgent to develop new strategies against infection. In this work, we evaluated an alternative antibacterial strategy based on the use of immune complexes (IC, confidential design), seeking to activate the intracellular response of macrophages infected and to affect the survival of the intracellular bacterium. **Methods.** The viability of intracellular and extracellular *P. salmonis* when infects SHK-1 cells were quantified post treatment with IC at 72 and 120h post-infection. Quantification of *P. salmonis* was done by absolute quantification by qPCR directed to 16S rDNA gene and by serial dilutions of colony forming units (CFU) in CHAB agar. Macrophages intracellular response was evaluated by determination of the oxidative burst levels by measurement of intracellular superoxide ion by the NBT-assay in SHK-1 infected and treated with IC. **Results.** The stimulation with IC in macrophages infected with *P. salmonis* decreases the cytopathic effect caused by the bacterium and decreases the viable bacterial load within SHK-1 cells. The intracellular response was observed slightly induced when macrophages infected by *P. salmonis* were stimulated with IC reflected by an increased anion superoxide levels. **Conclusion.** The results suggest that the stimulation with immune complex increased the host antimicrobial activity and possibly affects the viability of *P. salmonis* when infects Atlantic salmon macrophages.

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CLP3-44

Two novel chaperone-usher fimbriae of enterotoxigenic *Escherichia coli* as adherence determinants

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Enterotoxigenic *Escherichia coli* (ETEC) are a leading cause of diarrhea worldwide. ETEC represent a diverse set of *E. coli* producing the heat-labile toxin and/or the heat stable toxin. Colonization of the small bowel is a key step on the ETEC infection mechanism which is achieved by production of fimbrial colonization factors (CFs). Most of the CFs are assembled at the outer membrane by the chaperone-usher pathway (CU). Characterization and determination of the frequency of CFs among ETEC strains worldwide are crucial for the development of therapies. However, 15-30% of the ETEC isolates obtained from diarrhea cases are negative CF detection, suggesting presence of unknown structures. Recently, by performing comparative genomics analyses of CF-negative ETEC strains, we identified seven loci encoding putative CU-CFs of the gamma-2 family. In order to determine the role of two of these loci, *crs* and *ctg*, we generated mutant strains by allelic exchange in ETEC strains. ETEC strain 100664 lacking *crs* showed a reduced adherence capacity compared to the wild type but, in contrast, knocking out of *ctg* in ETEC strain 9343a did not cause the same effect. The goal of this work was to determine if the expression of *crs* and *ctg* in laboratory strains could direct the assembly of functional fimbriae. Both loci were cloned into the pEZ-BAC vector, and introduced into *E. coli* HB101 or *E. coli* DH10B. SDS-PAGE analysis of heat-extracted surface proteins allowed us to recognize bands present in the extracts of recombinant strains but absent in the extracts of the host strains harboring the empty vector. For both cases, bands are very close to the predicted sizes of the mature putative major structural subunits CrsH and CrsA (18kD approximately). Furthermore, analysis of both recombinant strains by transmission electron microscopy, allowed us to identify fimbrial structures of 7nm in diameter, absent in the control host strains. Finally, recombinant strains harboring *crs* and *ctg* were able to attach Caco-2 cells in a significantly higher level compared to the host strains carrying the empty vector. In summary, we are adding two novel CFs to the long list of ETEC adherence determinants.

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CLP3-45

Pathogenic and non-pathogenic *Escherichia coli* strains modulate the stress granules assembly

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Non-pathogenic *Escherichia coli* strains, one of the most abundant microorganisms in the gut microbiota, have developed a symbiosis relationship with their host to regulates immunological response. In contrast, Enterotoxigenic *Escherichia coli* (ETEC), the main etiologic agent in diarrhea morbidity and mortality in children under 5 years in developing countries, has developed mechanisms to reduce it immune-activator effect and carry out an effective infection. Following infection, the host cell initiates the shut-off of protein synthesis and stress granules (SGs) assembly. The assembly of SGs is generally mediated by the phosphorylation of translation initiator factor 2α (eIF2α). In this study, we evaluated the ability of an ETEC strain (ETEC 1766a, producing heat stable toxin, heat labile toxin and coli surface antigen 23) and a non-pathogenic *E. coli* strain (*E. coli* HS) to induce stress granules assembly, even in response of exogenous stresses. We found that infection of Caco-2 cells with both *E. coli* strains did not induce SG assembly. Furthermore, *E. coli*-infected Caco-2 cells subjected to treatment with sodium arsenite showed the ability to inhibit SG assembly. This effect was mediated by the impairment of eIF2α phosphorylation. Understanding how bacteria counters host stress responses will lay the groundwork for new therapeutic strategies to bolster host cell immune defences against these pathogens.

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CLP3-46

Effect of carbon source and oxygenation on the growht of *Janthinobacterium lividum* MTR1410 and the production of violacein, a pigment with potential application in aquaculture

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Background. *Janthinobacterium lividum* is a gram negative bacteria characterized by planktonic and sessile growth. This bacterium also produces a secondary metabolite, called violacein, a violet pigment which presents various properties of biotechnological interest: antimicrobial activity, antioxidant, anticancer, immunomodulatory, antiparasitic, among others, with potential application in aquaculture. In static cultures, after 24 hours of culture, violacein occurs in cells that form a dense biofilm at the air-liquid interface. So the availability of oxygen and nutrients can be important in the production of this pigment. In order to understand aspects of the synthesis of violacein in *Janthinobacterium lividum*, we hypothesized that its production would be modulated by the carbón source and by the administration of oxygen. **Methods.** In this work was used the strain *Janthinobacterium lividum* MTR1410, previously isolated in the laboratory. Identification of violacein was performed by HPLC, GC/MS and nuclear magnetic resonance (NMR). Violacein production was quatified by UV absorbance and HPLC. To determine the effect of the carbon source on the production of violacein, were used the media Luria Bertani and Nutritive supplemented with glucose and 0,2% glycerol. Cultures were performed at 20 °C in agitation. The effect of oxygenation on the production of violacein was determined by corelating the biochemical oxygen demand and the production of violacein in flasks with different volumes of culture médium. **Results** obtained by nuclear magnetic resonance (NMR) indicate that *Janthinobacterium lividum* MTR1410 generates more than one violacein isoform. On the other hand, the production of violacein was favored in culture in nutritive medium supplemented with glycerol in agitation. Regarding the effect of oxygenation on the production of violacein, it was posible to observe that oxygen increases the production of violacein. In **conclusion** *Janthinobacterium lividum* MTR1410 produces violacein in a manner dependent on the source of carbon and oxygen present under culture conditions, in amounts comparable to other strains producing violacein.

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CLP3-47

Effect of NatControl TM on immunity markers gene expression and the protection against in vitro infection with *Piscirickettsia salmonis*

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Background. NatControl™ is a special combination of plant extracts from the *Acanthaceae* family, standardized in specific labdane diterpenoid. Its immunostimulating properties have been studied in several models, but their effect on fish has been little studied. Therefore, this study aims to determine the *in vitro* properties of the extract in SHK-1 macrophagic cell line from *Salmo salar*. **Methods.** The effect of NatControl™ on the expression of innate immunity markers genes associated with antibacterial and antiviral mechanisms in SHK-1 cells (derived from *Salmo salar* head kidney) was evaluated. For this, a stimulation kinetics was performed with the product and the levels of IL-12 and IFN-I were quantified by RT-qPCR. Also, the protective effect of this formulation was evaluated by an *in vitro* challenge with *Piscirickettsia salmonis* strain LF-89 (10^4 and 10^6 bacteria / mL). The percentage of cytotoxicity was calculated by a colorimetric assay that measures LDH enzyme release into the culture medium of the treated and infected cells. **Results.** Our results show that 5 nM and 10 nM NatControl™ exhibit the highest effect on the expression of IL-12 and IFN-I in SHK-1 cells at 2 h and 12 h stimulation times. Also, against the challenge with *P. salmonis*, a protective effect was noticed on cells pretreated for 6 h with NatControl™, lasting until day 20 post-infection. It should be noted that the cells treated with the respective NatControl™ treatments presented negative values of toxicity at the beginning of the experiment, which could be due to a protective effect of the products on cell viability. **Conclusion.** We suggest a potential immunostimulating effect of NatControl ™ on SHK-1 cells and a protective effect against infection with *P. salmonis*. Further studies need to be performed in order to assess the immunostimulating and protective effect of this compound in fish models in response to infections with *P. salmonis*.

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Our special thanks to HP ingredients for giving us samples of NatControl ™

CLP3-48

Virulence and pathogenic effect of Influenza A(H1N1)pdm09 genetic variants with different plaque phenotypes

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The H1N1pdm09 virus responsible for the human influenza A pandemic in 2009 is a triple reassortant virus containing swine, human and avian genes. Genetic variations within a virus population can play an important role in its replicative capability and the outcome of viral infection. We identified and isolated two variants of the A/Nethetherlands/602/2009 (Neth/602) H1N1pdm09 strain, which showed a large (BP) and small (SP) plaque phenotypes, respectively. Both viral variants grew to high titers and produced cytopathic effect *in vitro* in MDCK cells. The SP virus demonstrated a faster replication kinetic at early stages of a multicycle growth curve, reaching higher titers at 32 hours before plateauing at similar level 48 hrs post infection (hpi) in MDCKs. When C57BL/6 mice were infected with both variant, the SP virus produced increased weight loss as compared to the BP virus. To elucidate the genetic differences of both variants responsible for their plaque and virulence phenotype, we sequenced their full genome. The viral variants contained four mutations as compared to the original Neth/602 isolate. Two of these mutations were present in the Hemagglutinin (HA-G172E) and Nucleoprotein (NP-G431R) proteins of the BP variant. The SP virus had mutations in the Polymerase Basic protein 2 (PB2-S155C) and in the Neuraminidase (NA-I407V). Utilizing an *in vitro* minigenome assay to investigate replication activity, we found a higher polymerase activity when the NP-431R from the BP virus was used in comparison to both WT and SP-PB2S155C proteins, suggesting that the polymerase activity might be responsible for the BP plaque phenotype. We used reverse genetics to generate recombinant viruses harbouring the BP and SP mutations, which showed the respective plaque size phenotypes at 38 hpi. While all SP viruses comparatively produced more disease in mice, SP-PB2 mutant demonstrated the highest virulence out of all the viruses. Additionally, the replicative capacity of these viruses in human tracheobronchial epithelial (HTBE) cells also showed increased kinetics of the SP viruses, which correlate with increased disease in the mouse model. These analyses might aid us to evaluate the virulence of clinical isolates with differential plaque phenotypes and genotype obtained from influenza A patients.

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CLP3-49

Construction of a luciferase sensitive to cleavage by the IPNV protease

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Infectious pancreatic necrosis virus (IPNV) is a disease that produces important losses in Salmon farming. IPNV is a small Birnavirus, icosahedral, non-enveloped particle that contains two segments of double stranded RNA (segment A and B). Segment A has two open reading frames. The mayor open reading frame encodes a 106 kDa polyprotein that is processed to VP2, VP3 and VP4. VP2 and VP3 are capsid structural proteins while VP4 is a protease. Segment B encodes VP1 a RNA polymerase RNA's dependent. VP4 is an autolytic serine protease that cleaves the polyprotein in six different positions but also acts *in trans*. The objective of this work was to incorporate the recognition site of the protease in the luciferase polypeptide by site specific mutagenesis to measure the loss of luciferase activity upon exposure to the protease. We found that in partly purified preparations of the luciferase mutant, activity decreases after incubation with extracts from *E. coli* BL21 (DE3) producing the protease of IPNV. Our results suggest that the modified luciferase could be use as a detection system for IPNV infection.

CLP3-50

Phenotypic characterization of shiga toxin-producing *Escherichia coli* (STEC) from different origins in Chile

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Background. Shiga toxin-producing *Escherichia coli* (STEC) is a public health concern due to its association to foodborne transmitted human disease. It mainly affects children under 5 years old and the elderly who can suffer hemolytic uremic syndrome as a severe complication. Multiple STEC serotypes have been associated to disease, but *E. coli* O157:H7 is the most studied. Many STEC detection methods were developed based on O157:H7 biochemistry; however, STEC is a highly heterogeneous group, and biochemical characteristics may differ among isolates complicating STEC isolation. The aim of this study was to characterize biochemical features of STEC isolated in Chile from different origins to determine whether isolation methods based on STEC O157:H7 will work for Chilean isolates. **Methods.** We characterized 99 STEC isolates from different origins: ground beef (40), cattle (53), wild birds (2), cheese (3) and a clinical case (1). Isolates were previously identified as STEC by PCR for *E. coli*, *stx*$_1$ and *stx*$_2$. Biochemical tests were run for sorbitol fermentation (Sorbitol McConkey agar (SMAC)), tellurite resistance (SMAC supplemented w/2.5 µg/ml tellurite), β-glucuronidase activity (TBX agar) and hemolysis (washed sheep blood/enterohemolysin agar). **Results.** From 99 studied isolates, 86.9% (86) fermented sorbitol; 95.9% (95) displayed β-glucuronidase activity; 66.7% (67) were sensitive to tellurite. This characteristic differs from STEC O157:H7 typical phenotypic profile (non-sorbitol fermenting, non β-glucuronidase activity, and resistant to tellurite). Also, 60.6% (60) of isolates were enterohemolysin producer (EHEC-Hly) and 22.2% (22) caused α-hemolysis suggesting these strains carry some virulence factors. We did not detect profile differences among strains isolated from different matrices. **Conclusion.** STEC isolated in Chile have different biochemical profiles than *E. coli* O157:H7. Selective media based on STEC O157:H7 biochemical characteristics seem unsuitable for isolating and identifying STEC found in Chile in different origins, especially those containing tellurite.

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CLP3-51

Use of modified lactic acid bacteria (LAB) for control disease in salmonids

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Aquaculture is one of the most important chilean economic resources. However, the production of different species is exposed to different problems, including infectious diseases. The antibiotics administration and vaccines are the most utilized resources for the control of these diseases, however, they generate unwanted side effects. It has been proposed as alternative treatment to control pathogens the use of probiotics (lactic acid bacteria (LAB)) that confer benefits to the health of the host. In this work, we present the protective effects of immunostimulant BAL against bacterial pathogens in salmonids. Mortality and the bacterial load were recorded for two weeks. The results showed that salmonids treated and challenged with *F. psychrophilum* had an increased survival respect to salmonids untreated. On the other hand, treated salmonids challenged with *P. salmonis* had a survival rate 3.75 times better than untreated salmonids. Surviving fish showed lower or no bacterial load. In conclusion, the results show that modified LABs would be an effective method to prevent mortalities associated with bacterial pathogens affecting fish of aquaculture importance.

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CLP3-52

The immunity to the respiratory syncytial virus is impaired by hypothyroxinemia during gestation

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The human Respiratory Syncytial Virus (hRSV) is the major cause of lower respiratory tract illness in infants and young children worldwide. Previous reports indicate that alterations in the hormonal levels can affect the immune system, specifically, the thyroid hormones (THs) could contribute to the immune response against viral infections. Data from our laboratory showed that mice gestated in hypothyroxinemic mothers display a reduced population of activated CD8+ T cells as compared to mice gestated in euthyroid mothers (n= 9; $p<0.5$). Taking into account that CD8+ T cell response is essential against viral infection. We decided to evaluate whether the disease caused by hRSV is altered in the progeny gestated during low levels of T_4 hormone. Induction of hypothyroxinemia (HPX) during gestation resulted in decreased levels of maternal T_4 (n= 9; $p< 0.05$). Mice gestated in HPX, mice gestated in euthyroidism (Eut) or mice gestated in HPX plus T_4 hormone (HPX+T_4) were infected intranasally with hRSV. We evaluated loss of weight, viral loads (qRT-PCR), expression and production of cytokines (qRT-PCR and ELISA, respectively) and cellular infiltration by cytometry flow. We observed that mice gestated under HPX condition showed a greater weight loss (n= 9;$p<0.5$) and increased viral loads in the lungs (n= 9; $p<0.1$), as compared to mice gestated by euthyroid mothers. In addition, the offspring gestated by HPX mothers showed reduced secretion of interferon gamma (IFN-γ) (n= 9;$p<0.5$), but without changes in IL-10 and TNF-a production, correlating with fewer infiltrating $CD8^+$ T cells in the lungs and bronchoalveolar lavage fluid (BALF) (n= 9; $p<0.001$) without affect the infiltration of $CD4^+$ T cells and neutrophils after hRSV infection. These data support the notion that an alteration in the levels of THs can modulate infection by hRSV, increasing the susceptibility to the pathology caused by this virus.

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CLP3-53

Loop-mediated isothermal amplification (LAMP) as a powerfull tool for a rapid diagnostic of bacterial pathogens in farmed salmon fish

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Chile is the second largest producer of salmonid fishes after Norway, however the emergence of infectious disease poses a serious threat to the productivity. The major bacterial pathogens that affect the Chilean salmon industry are *Piscirickettsia salmonis* and *Renibacterium salmoninarum*, etiological agents of the Piscirickettsiosis and the bacterial kidney disease (BKD) respectively. In fact, both diseases produce annual economic losses between US$ 700-800 million and together are responsible for 82% of antibiotic therapies performed in the marine stage. Due the economic impact of both bacterial diseases is absolutely necessary the development of a rapid, sensible and field applicable diagnostic strategy in order to achieve preventive detection of both pathogens. In this work was applied and optimized an innovative PCR-derived technique, called Loop-mediated Isothermal Amplification (LAMP) for *P. salmonis* and *R. salmoninarum* diagnostic. Using in silico analyses of *P. salmonis* and *R. salmoninarum* genomes were selected specific molecular markers as targets for LAMP amplification in each pathogen. Primers sets (6 for each target) were designed using the PrimerExplorer Software and then all amplification conditions were experimentally optimized (temperature, time and reagent concentration). The high sensitive of LAMP amplification was validated using DNA dilutions of known concentrations for each pathogen. Finally, the specificity of the best primer set was tested against different DNA and RNA sources, including other bacterial and virus pathogens, *Salmo salar* microbiota, among others. Our results show that the LAMP reaction was optimized for 30 minutes at 60ºC for each genetic marker. Aditionally, the primers designed shown to be highly sensitive, being able to detect being able to detect 101 copies of bacterial, similar to the qPCR. The LAMP reaction was totally specific for each pathogen, not showing cross-reaction with DNA from other sources. Finally, the technique was validated with field samples, using a simple DNA purification system (tissue disruption and dilution) were obtained reproducible amplification results. In conclusion, our results show LAMP as a promising alternative for a rapid, sensitive, efficient and potentially field-applicable diagnostic tools for *P. salmonis* and *R. salmoninarum* in non-symptomatic carrier fishes (preventive detection).

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