SCIENTIFIC PROGRAM

ABSTRACTS & POSTERS

2nd edition

of the international meeting

BioAqua 2022

FROM BASIC RESEARCH TO APPLIED SCIENCE

AQUATIC BIOTECHNOLOGY

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March 27th to 31th, 2022

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BioAqua 2022
FROM BASIC RESEARCH TO APPLIED SCIENCE

SCIENTIFIC PROGRAM
ABSTRACT BOOK

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BioAqua 2022
FROM BASIC RESEARCH TO APPLIED SCIENCE

CIGB CENTRO DE INGENIERÍA GENÉTICA Y BIOTECNOLOGÍA

CONTENT

BioAqua 2022
FROM BASIC RESEARCH TO APPLIED SCIENCE

CIGB CENTRO DE INGENIERÍA GENÉTICA Y BIOTECNOLOGÍA

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GENERAL PROGRAM

Sunday **March 27th**	Monday **March 28th**	Tuesday **March 29th**	Wednesday **March 30th**	Thursday **March 31th**
8:00-14:00 **Transfer to hotel**	8:30-9:30 **Key Lecture**	8:30-9:30 **Key Lecture**	8:30-9:30 **Key Lecture**	8:30-9:30 **Final Remarks**
14:00-17:00 **Check in – Registration**	9:30-11:00 **Oral Session**	9:30-11:00 **Oral Session**	9:30-11:00 **Oral Session**	
Lunch	11:00-11:30 **Coffe Break**	11:00-11:30 **Coffe Break**	11:00-11:30 **Coffe Break**	
	11:30-13:30 **Oral Session**	11:30-13:30 **Oral Session**	11:30-13:30 **Oral Session**	11:00-12:00 **Check out**
	13:30-15:00 **Lunch**	13:30-15:00 **Lunch**	13:30-15:00 **Lunch**	13:30-15:00 **Lunch**
	15:00-17:00 **Poster Session**	15:00-17:00 **Touristic Excursion** Dolphinarium	15:00-17:00 **Poster Session**	15:00-17:00 **Departure**
19:00-23:30 **Welcome Dinner & Party**	20:00 **Free night**	20:00 **Free night**	21:00-23:00 **Beach party**	

SCIENTIFIC PROGRAM

Monday, March 28

Key Lecture 8.30 am-9.30 am

Title: Cuban biotechnology, advances and upcoming challenges

By Dr. Mario Pablo Estrada, Center for Genetic Engineering and Biotechnology, Havana, Cuba

Session chairmans: Dr. Carolina Tafalla/Dr. Mario Pablo Estrada

9:30-10:00 Novel insights into the role of IgD in teleost fish. By Dr. Carolina Tafalla. CISA-INIA-CSIC, Spain

10:00-10:30 Simultaneous analysis of 12 distinct populations of leukocytes following pathogenic challenge in rainbow trout (*Oncorhynchus mykiss*). By Dr. Alvaro Fernández Montero, UPENN, USA

10:30-11:00 Study of the role of Immunoglobulin T in the humoral immune response in Nile tilapia (*Oreochromis niloticus*). By Janet Velázquez. CIGB, Cuba

11:00-11:30 Coffee Break

11:30-12:00 Developing antibody reagents to assess immune functions in fish. By Dr. Tania Rodríguez-Ramos. Waterloo University, Canada

12:00-12:30 *Bacillus subtilis* spores as a combined adjuvant-delivery system for new generation vaccines in aquaculture. By Dr. Patricia Díaz -Rosales, CISA-INIA-CSIC, Spain

12:30-13:00 Transcriptome modulation of *Salmo salar* immunized with *Caligus rogercresseyi* vaccine prototype: a host-parasite interaction. By Antonio Casuso, Universidad de Concepción, Chile

13:00-13:30 Whole-genome resequencing of the sea lice *Caligus rogercresseyi* reveals novel genomic markers associated with pharmacological resistance. By Dr. Gustavo Núñez-Acuña, Universidad de Concepción, Chile

13:30-15:00 Lunch

15:00-17:00 Poster session

SCIENTIFIC PROGRAM

Tuesday, March 29

Key Lecture 8.30 am-9.30 am

Title: Teleost swim bladder, an ancient air-filled organ that elicits anti-viral innate and adaptive mucosal immune responses. By Dr. J. Oriol Sunyer, University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, Philadelphia, PA, USA.

Session chairmans: Dr. Oriol Sunyer/Dr. Rebeca Martínez

9:30-10:00 Advances in sea lice genomics. By Dr. Cristian Gallardo-Escárate. Universidad de Concepción, Chile

10:00-10:30 RNAseq data analysis of *Oreochromis niloticus* tilapias treated with Growth Hormone Releasing Peptide-6. By Dr. Ricardo Bringas, CIGB, Cuba

10:30-11:00 Whole-genome expression approach for transcriptome analysis to understand Atlantic salmon sea water adaptation. By Dr. Valentina Valenzuela-Muñoz, Universidad de Concepción

11:00-11:30 Coffee Break

11:30-12:00 Meta-analysis: a powerful tool in aquaculture. By Dr. Amílcar Arenal, Universidad de Camagüey, Cuba

12:00-12:30 Evading the antibodies – insights into antigenic variability in the myxozoan parasite *Sphaerospora molnari*. By Dr. Tomáš Korytář, Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic

12:30-13:00 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) boosts antimicrobial defenses in teleost fish: development of an oral formulation. By Dr. Yamila Carpio, CIGB, Cuba

13:00-13:30 Immunomodulatory properties of Smolstatin, a cysteine protease inhibitor of the myxozoan parasite *Sphaerospora molnari*. Dr. Amparo Picard-Sánchez, Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic

13:30-15:00 Lunch

15:00-17:00 Touristic Excursion

SCIENTIFIC PROGRAM

Wednesday, March 30

Key Lecture 8.30 am-9.30 am

Title: Anti-microbiota vaccines, concepts and applications

By Dr. Alejandro Cabezas Cruz, The Joint Research Unit for Molecular Biology and Parasitic Immunology (UMR BIPAR), (INRAE- Anses- EnvA), Paris, France

Session chairmans: Dr. Cristian Gallardo/Dr. Yamila Carpio

9:30-10:00 Administration of growth hormone secretagogue molecule in fishes: implications for sustainable aquaculture and conservation. By Dr. Rebeca Martínez, CIGB, Cuba

10:00-10:30 The sea lice microbiota: a potential threat for salmon aquaculture. By Dr. Diego Valenzuela-Miranda, Universidad de Concepción, Chile

10:30-11:00 Potential effect of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) with its variants against *Flavobacterium psychrophilum*, *Flavobacterium columnare*, and *Aeromonas salmonicida*. By Dr. Lowia Al-Hussinee, Waterloo university, Canada

11:00-11:30 Coffee Break

11:30-12:00 Covalent modifications of Cm-p5, a peptide derived from marine mollusk (*Cenchritis muricatus*), a path towards the development of new pharmacological viable AMPs. Dr. Fidel E. Morales Vicente, CIGB, Cuba

12:00-12:30 Effect of a growth hormone secretagogue peptide (GHRP-6) on the hormonal control of reproduction and the expression of genes linked to the immune system in gills and spleens of tilapia (*Oreochromis niloticus*). By Adrian Rodriguez, CIGB, Cuba

12:30-13:00 The response of aquatic invertebrates to immune stimulation by dsRNA and PACAP-38. By Emma Monod, Waterloo University, Canada

13:30-15:00 Lunch

15:00-17:00 Poster session

KEY LECTURES

Title: Cuban biotechnology: advances and upcoming challenges

Author: Dr. Mario Pablo Estrada, Director of Agricultural biotechnology, Center for Genetic Engineering and Biotechnology, Havana, Cuba

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Abstract:

Since 1959, the Cuban government has as central objective the development of the national science. To that end, it established multiple educational programs and granted important budgetary allocations toward the formation of qualified personnel and necessary research, development and production facilities. In 1992, the scientific pole was established comprising more than 50 institutions with a structure to operate in a closed cycle, from research to product exportation. The experience of Cuban biotechnology can be considering a success taking into account the amount of biopharmaceuticals and vaccines developed, the impact on public health, patent registration and exports. The interesting aspect of Cuban biotechnology is its specialization in preventive vaccines produced locally. In December 2012, the industry transformed as biotechnology companies became part of the BioCubaFarma, one of the high level organizations for enterprise management. The Cuban biopharmaceutical industry has been key in combating the coronavirus pandemic. Most of the products are domestic and have been effective, including the development of recombinant vaccines to immunize the population. Agrobiotechnology has been also an area of development and success with unique vaccines against bovine ticks or classical swine fever virus, to mentioned just two examples. To continue the Cuban biotech development will be necessary to continue generating innovative and competitive products, diversifying the market in the export process and the injection of financial resources. Biotechnology is a strategic sector in terms of multidimensional aspect of Cuba's development aspirations for 2030.

KEY LECTURES

Title: Teleost swim bladder, an ancient air-filled organ that elicits anti-viral innate and adaptive mucosal immune responses

Author: J. Oriol Sunyer

Affiliations: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, Philadelphia, PA, USA.

E-mail: sunyer@vet.upenn.edu

Abstract:

Air-filled organs (AOs) emerged approximately 400 million years ago in early ray-finned fishes and are a defining and crucial feature for the survival of bony vertebrates in water. Throughout the evolution of these species, AOs underwent important adaptive changes in response to different environmental pressures. Interestingly, instead of evolving into lungs like those of terrestrial vertebrates, AOs in teleosts evolved into swim bladders (SB). In these species, the SB is most well-known for its role in buoyancy control although in some species it can also play auxiliary functions in respiration, sound production and hearing. However, when vertebrates colonized terrestrial ecosystems, basal lobe-finned fishes (e.g., lungfish), the AOs evolved into lungs that were functionally similar to those of tetrapods in their gas exchange roles. Recently, the evolutionary relationships between AOs have gained significant attention. In that regard, recently reported from genetic data strongly suggests a common ancestry between lungs and SB, and therefore both organs likely originated in parallel from primitive lungs in the last common ancestor of early ray-finned fish. While it is clear that lungs of tetrapods are known to play a key role in immunity through their mucosal-associated lymphoid tissue (MALT), nothing is known about the potential roles of the SB in adaptive immunity. Here we hypothesized that due to the common ancestral origins of lungs and SB from primitive lung-like organs, the study of the SB immune functions could shed light into the evolutionary origins of AOs MALT and its primordial roles in immune defense and microbiota homeostasis.

Thus far, mucosal immune responses in the MALT of lungs have mainly been described in mammals and, to a lesser extent, in birds, where IgA plays a pivotal role in mucosal immunity. Teleost fish also contain MALTs in several mucosal organs, and our group has previously shown that teleosts contain secretory IgT (sIgT), the most ancient mucosal immunoglobulin described thus far. From a morphological perspective (Fig. 1), the teleost SB has a fairly simple structure, and it is comprised of an air-filled thin, translucent membrane that is connected to the esophagus (Fig. 1A shows the SB of rainbow trout, our model species). Given the mucosal nature of the SB surface and the common evolutionary ancestry between lungs and SB, we hypothesized that AOs in both primitive and modern bony vertebrates must have evolved analogous molecular mechanisms for mucosal immunity. This study was therefore undertaken to examine whether the AOs of an aquatic species (i.e, teleost SB) could fulfill immune roles.

KEY LECTURES

In this study we demonstrate that the SB contains a *bona fide* MALT and plays a previously unrecognized role in mucosal immunity. In order to evaluate the capacity of the SB to behave as an immune responsive tissue, we established an infection model with infectious hematopoietic necrosis virus (IHNV) which was inoculated to fish via intra-SB cavity injection. We found that IHNV infection elicited a strong innate immune response in the SB as shown by the upregulation of key genes involved in innate and anti-viral immune responses. Moreover, re-infection with the virus induced local IgT^+ B cell proliferation in the fish SB. Importantly, we demonstrate the production of IHNV-specific sIgT within the SB and show the *in vitro* capacity of specific sIgT in viral neutralization, thus exposing a previously unrecognized effector function of sIgT in viral defense. The key role of SB sIgT in antiviral immunity was further demonstrated by the use of fish selectively depleted of IgT, using a strategy recently described by our groups. We found that the fish that survived IHNV challenge achieved significant protection against reinfection, whereas the SB viral load IgT-depleted fish increased tremendously upon reinfection. These findings constitute the first demonstration of the occurrence of innate and adaptive mucosal immunity in the AOs of an aquatic species, while also exposing a previously unrecognized key role of sIgT in antiviral defense. Here we also show that similar to other teleost mucosal surfaces, the SB contains a significant microbiota population that is prevalently coated by sIgT, and to a much lesser degree by IgM and IgD. Interestingly, the amount of microbiota in the SB was the lowest of all fish MALTs, a situation that resembles that of the lungs from tetrapods which contain a very small amount of microbiota when compared to other mucosal surfaces.

In addition to its well-known role in buoyancy control, our results reveal a previously unrecognized role of teleost SB in immunity. Moreover, our study demonstrates that vertebrate AOs and specialized mucosal Igs are part of an ancient partnership that predates the emergence of tetrapods. Critically, as the sIgT of fish is functionally analogous to mammalian sIgA, our findings indicate that mucosal immune responses in the SB and lungs of primitive and modern vertebrates respectively evolved through a process of convergent evolution.

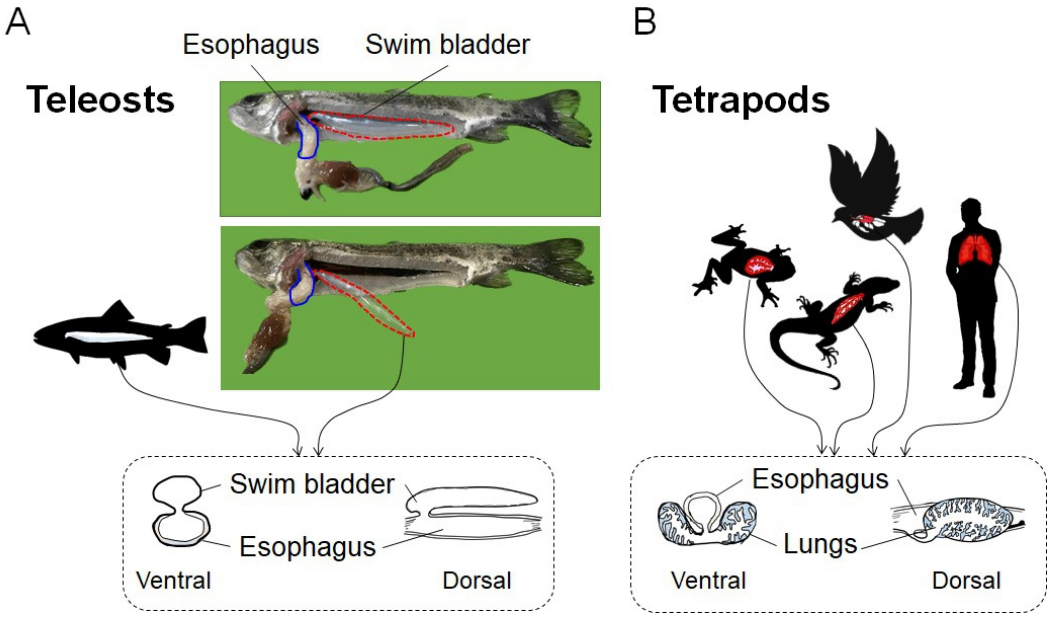

Fig. 1: Diagrammatic cross and longitudinal sections of the lung and SB in vertebrates.

ORAL PRESENTATIONS

Notice:
Abstracts are indexed by title in alphabetical order

Administration of growth hormone secretagogue molecule in fishes: implications for sustainable aquaculture and conservation. By Dr. Rebeca Martínez, CIGB, Cuba

Advances in sea lice genomics. By Dr. Cristian Gallardo-Escárate. Universidad de Concepción, Chile

Bacillus subtilis spores as a combined adjuvant-delivery system for new generation vaccines in aquaculture. By Dr. Patricia Díaz -Rosales, CISA-INIA-CSIC, Spain

Covalent modifications of Cm-p5, a peptide derived from marine mollusk (Cenchritis muricatus), a path towards the development of new pharmacological viable AMPs. Dr. Fidel E. Morales Vicente, CIGB, Cuba
Developing antibody reagents to assess immune functions in fish. By Dr. Tania Rodríguez-Ramos. Waterloo University, Canada

Effect of a growth hormone secretagogue peptide (GHRP-6) on the hormonal control of reproduction and the expression of genes linked to the immune system in gills and spleens of tilapia (Oreochromis niloticus). By Adrián Rodríguez, CIGB, Cuba

Evading the antibodies – insights into antigenic variability in the myxozoan parasite Sphaerospora molnari. By Dr. Tomáš Korytář, Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic

Immunomodulatory properties of Smolstatin, a cysteine protease inhibitor of the myxozoan parasite Sphaerospora molnari. Dr. Amparo Picard-Sánchez, Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic

Metaanalysis: a powerful tool in aquaculture. By Dr. Amílcar Arenal, Universidad de Camagüey, Cuba

Novel insights into the role of IgD in teleost fish. By Dr. Carolina Tafalla. CISA-INIA-CSIC, Spain

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) boosts antimicrobial defenses in teleost fish: development of an oral formulation. By Dr. Yamila Carpio, CIGB

Potential effect of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) with its variants against *Flavobacterium psychrophilum*, *Flavobacterium columnare*, and *Aeromonas salmonicida*. By Dr. Lowia Al-Hussinee, Waterloo university, Canada

ORAL PRESENTATIONS

Notice:
Abstracts are indexed by title in alphabetical order

RNAseq data analysis of *Oreochromis niloticus* tilapias treated with Growth Hormone Releasing Peptide-6. By Dr. Ricardo Bringas, PhD

Simultaneous analysis of 12 distinct populations of leukocytes following pathogenic challenge in rainbow trout (*Oncorhynchus mykiss*). By Dr. Alvaro Fernández Montero, UPENN, USA

Study of the role of Immunoglobulin T in the humoral immune response in Nile tilapia (*Oreochromis niloticus*). By Janet Velazquez. CIGB, Cuba

The response of aquatic invertebrates to immune stimulation by dsRNA and PACAP-38. By Emma Monod, Waterloo University, Canada

The sea lice microbiota: a potential threat for salmon aquaculture. By Dr. Diego Valenzuela-Miranda, Universidad de Concepción, Chile

Transcriptome modulation of *Salmo salar* immunized with *Caligus rogercresseyi* vaccine prototype: a host-parasite interaction. By Antonio Casuso, Universidad de Concepción, Chile

Whole-genome expression approach for transcriptome analysis to understand Atlantic salmon sea water adaptation. By Dr. Valentina Valenzuela-Muñoz, Universidad de Concepción

Whole-genome resequencing of the sea lice *Caligus rogercresseyi* reveals novel genomic markers associated with pharmacological resistance. By Dr. Gustavo Núñez-Acuña, Universidad de Concepción, Chile

ORAL PRESENTATIONS

Title: Administration of growth hormone secretagogue molecule in fishes: implications for sustainable aquaculture and conservation.

Authors: Liz Hernández[1], Daniela Rodríguez[1], Anne Gaelle Lafont[2] ,Sylvie Dufour[2], Salima Aroua[3], Andrés Hurtado [4], Mario Pablo Estrada[5] and Rebeca Martínez [1]

Affiliations: [1]Metabolic Modifiers Project, Department of Animal Biotechnology, Center for Genetic Engineering and Biotechnology, Havana, Cuba, [2]Muséum National d'Histoire Naturelle, Sorbonne Universités, Research Unit BOREA, Biology of Aquatic
Organisms and Ecosystems, CNRS 7208, IRD207, UPMC, UCBN, Paris, France, [3]SEBIO , Universite du Havre, BP 1123, 76063 Le Havre, Cedex ,France, [4]Center for Reproduction of the Indigenous Ichthyofauna, Ciénaga de Zapata National Park, Matanzas, Cuba, [5]Director of Agricultural Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba.

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Abstract:

Aquaculture is an important socio-economic activity in many countries and is the fastest growing animal food production sector in the world. With more than 25,000 species, the fish of the class Actinopterygii represent the largest group of vertebrates, with a great diversity of species with biological, ecological and socio-economic importance. The study of the regulatory mechanisms of the life cycles and the health of the fish, allows the development of sustainable aquaculture, thus contributing to food security and the conservation of aquatic biodiversity. Ghrelin is a multifunctional hormone produced in the stomach cells and exerts its functions in different tissues. It is involved in processes such as appetite, metabolism and growth, as well as in the regulation of reproduction and immunity. In this work, growth stimulation results obtained in tilapia and sea bass larvae by the action of a ghrelin mimetic are presented. Also, it was demonstrated that the treatment with this molecule, stimulated the superoxide anion release in peripheral lymphocytes of Cuban gar. A nucleotide sequence of the Cuban gar (*Atractosteus tristoechus*) ghrelin was isolated.

ORAL PRESENTATIONS

Title: Advances in sea lice genomics

Author: Cristian Gallardo-Escárate

Affiliations: Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile

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Abstract:
The genomic era has increased the research effort to uncover how an organism's genome, and specifically the transcriptome, is modulated after interplaying with pathogenic microorganisms, pollutants, and environmental stressors. Next-generation sequencing technologies are currently utilized for the expression profiling and the discovery of novel gene signaling pathways that are relevant for biological processes such as reproduction, growth, immune response, and metabolism. The scientific information derived from functional genomics studies highlighted the relevance of incorporating omics approaches in areas such as fish production, where prevalent pathogens threaten the development of sustainable aquaculture. Here, the sea lice *Caligus rogercresseyi* and *Lepeophtheirus salmonis* are marine ectoparasites and a primary concern for salmon aquaculture worldwide. In this context, genome research has intensively studied the transcriptome signatures of lice species in response to pesticides and environmental factors, giving relevant molecular knowledge. However, while this information was revealed by canonical gene pathways involved in xenobiotic metabolism, several studies suggest an active role of non-coding RNAs as key regulators. We reported the construction of chromosome-level genome for *C. rogercresseyi*, and how this molecular information is currently used in several research projects related to larval ecology, drug resistance and vaccine development to control the lice infestation in the salmon aquaculture.

Funding: ANID-Chile through the grant FONDECYT (#1210852), and FONDAP (#15110027).

ORAL PRESENTATIONS

Title: *Bacillus subtilis* spores as a combined adjuvant-delivery system for new generation vaccines in aquaculture

Authors: Félix Docando[1], Noelia Núñez-Ortiz[1], Gabriela Gonçalves[2,3], Cláudia R. Serra[2,3], Eduardo Gómez-Casado[4], Diana Martín[1], Beatriz Abós[1], Paula Arense[1], Aires Oliva-Teles[2,3], Carolina Tafalla[1], Patricia Díaz Rosales[1]

Affiliations: [1]Fish Immunology and Pathology Group, Animal Health Research Centre (CISA-INIA-CSIC), Valdeolmos-Alalpardo, Madrid, Spain, [2]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Matosinhos, Portugal, [3]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal, [4]Department of Biotechnology, National Agricultural and Food Research and Technology Institute (INIA-CSIC), Madrid, Spain

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Abstract:

Oral vaccines are highly demanded by the aquaculture sector, as they are non-invasive and can be easily administered to fish of various sizes and ages. However, most previous attempts to obtain effective oral vaccines for fish have failed. To revert this, it is important to design oral vaccines that can deliver the antigen to the most distant segments of the intestine and are able to circumvent intestinal tolerance, thus inducing an adequate immune response to the antigen.

Probiotics have been widely tested as oral adjuvants in mammals. Furthermore, these bacteria have a great potential to be engineered as delivery platforms for antigens. Among the probiotics commonly used in aquaculture, *Bacillus subtilis* is one of the most studied species due to its spore forming ability. The robustness of *B. subtilis* spores, their gene operability, safety and adjuvant properties, make them ideal to design effective oral vaccination strategies.

In this context, we have used different approaches to undertake an initial evaluation of the potential of *B. subtilis* as either an oral adjuvant or as an antigen delivery vehicle in rainbow trout (*Oncorhynchus mykiss*). For this, we have first compared the immunomodulatory potential of two different *B. subtilis* strains, studying the response of gut epithelial cells, gut explants or immune tissues after a single administration of the bacteria. Additionally, we have engineered a *B. subtilis* strain expressing the VP2 protein from infectious pancreatic necrosis virus (IPNV). After confirming that this novel strain retained its immunomodulatory properties, we have demonstrated that it was capable of eliciting a robust antibody response to the virus after a single intraperitoneal or oral administration. The obtained results provide valuable information regarding how *B. subtilis* can be used to design effective oral vaccines for use in aquaculture, either as a mucosal adjuvant or also as an antigen delivery platform.

ORAL PRESENTATIONS

Title: Covalent modifications of Cm-p5, a peptide derived from marine mollusk (*Cenchritis muricatus*), a path towards the development of new pharmacological viable AMPs

Authors: Fidel E. Morales Vicente,[1] Melaine González García,[2] Frank Rosenau,[3] Hilda Garay,[1] Ludger Ständker,[4] and Anselmo J. Otero-Gonzalez[2]

Affiliations: [1]Synthetic Peptides Group, Center for Genetic Engineering and Biotechnology, La Havana 10600, Cuba, P.O. Box 6162, La Habana 10600, Cuba, [2]Center for Protein Studies, Faculty of Biology, University of Havana, 25 and I, 10400 La Habana, Cuba, [3]Institute of Pharmaceutical Biotechnology, Ulm University, James-Frank-Ring N27, 89081 Ulm, Germany, [4]Core Facility for Functional Peptidomics, Ulm Peptide Pharmaceuticals (U-PEP), University Ulm, Faculty of Medicine, Ulm University, 89081 Ulm, Germany

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Abstract:

Sea and freshwater mollusks are promising organisms for the identification of AMPs given that their immune system mainly relies on innate response. However, classical AMPs, have rapid proteolytic degradation, low activity compared to traditional antibiotics, high sensitivity to ionic environment and significant toxicity, especially hemolytic effect. Cm-p5, a peptide derived from the marine mollusk *Cenchritis muricatus*, has a significant fungistatic activity against pathogenic *Candida albicans*, while exhibiting low toxic effects against cultured mammalian cell line. However, in a systemic candidiasis model in mice, intraperitoneal administration of Cm-p5 was unable to control the fungal kidney burden, probably due to its low metabolic stability and presence of residues that could be easily affected by ions. We design a disulfide bridged cyclic analogue of this peptide that has the double of anti-candida activity. Also, parallel and antiparallel dimers of Cm-p5 showed a moderate activity against Pseudomonas aeruginosa PA14. The three Cm-p5 derivatives inhibited a virulent extracellular strain of Mycobacterium tuberculosis, in a dose-dependent manner. Moreover, they inhibited HSV-2 infection in a concentration-dependent manner, but had no effect on infection by the ZIKV or pseudo-particles SARS-CoV-2. Only at concentrations far superior to the therapeutic windows, the three new Cm-p5 derivatives showed toxicity on some eukaryotic cells tested. Remarkably, these novel analogues are potent biofilm inhibitors of the super bug *C. auris*. In conclusion, the covalent modification by disulfide bridge cyclization/dimerization, increase antifungal, antibacterial, antiviral and anti-biofilm activities of Cm-p5, together with an expected increase in metabolic stability and reduced influence of ionic environment.

ORAL PRESENTATIONS

Title: Developing antibody reagents to assess immune functions in fish

Authors: Tania Rodríguez-Ramos[1], Aaron Frenette[1], Tania Rodríguez Cornejo[1], Quinn Abram[2], Norm Tran[1], Shawna L. Semple[3], George Heath[1], Xiaoqing Dang[1], Victoria M. Gardiner[1], Jack Iwanczyk[4], Jessy Rix[5], Kurt A. Gamperl[6], Brian Dixon[1].

Affiliations: [1]University of Waterloo, Waterloo, Canada, [2]McGill University, Montreal, Canada, [3]University of Alberta, Alberta, Canada, [4]Cedarlane Laboratories Limited, Burlington, Canada, [5]Somru, BioScience Inc., Charlottetown, Prince Edward Island, Canada, [6]Ocean Science Center. Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

E-mail: tania.rodriguez-ramos@uwaterloo.ca

Abstract:

Advances in the knowledge on immune functions in mammals have been possible due to the development and validation of reagents that allow the detection and quantification of immunomarkers at protein level. Transcript-based assays are useful tools as a first approach to the understanding of immune molecules and mechanisms, but they must be followed by functional studies to evaluate the abundance and bioactivity of proteins involved in immune responses. Several transcriptomic studies have been performed in teleost fishes, however the scarce availability of antibodies to key proteins has kept a knowledge gap between the changes that we obtain at transcript level and how these changes would affect or not the presence and abundance of proteins. The development, standardization, and validation of antibody-based assays in fish faces many challenges. The antibody reagents used in these assays need to have a high sensitivity since most of the proteins involved in fish immune responses are produced at the pg/mL range, and they also need to be highly specific to avoid misled conclusions. Herein, we present results and challenges in the development and validation of polyclonal antibody-based assays to detect and quantify salmonid immune proteins (IL-1β, IFN1, IFNγ, CIRBP) in the context of identifying and evaluating immunomarkers to facilitate more efficient disease management in aquaculture.

ORAL PRESENTATIONS

Title: Effect of a growth hormone secretagogue peptide (GHRP-6) on the hormonal control of reproduction and the expression of genes linked to the immune system in gills and spleens of tilapia (*Oreochromis niloticus*).

Authors: Adrián Rodriguez-Gabilondo[1*], Daniela Felipe-Moro[1]. Liz Hernández[1],. Ricardo Bringas-Pérez[4],. Frank Torres-Valdés[4],. Hamlet Camachot[2], Daniel Palenzuela[2],. Janet Velazquez[3], Antonio Morales[1], Fidel Herrera[1], Osmany Rodrigo[1]. Mario P Estrada [5], Rebeca Martínez[1]

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Abstract:

Aquaculture is the fastest growing food production sector in the world. However, one of the main challenges for the development of aquaculture is the control of the reproductive function and the reduction of infectious diseases of the species. In this scenario, the use of immunostimulant molecules that stimulate reproductive processes is attractive. The innate immune system of fish is considered the first line of defense against a wide spectrum of pathogens. Among the molecules involved in this response are antiviral factors and enzymes with important effector functions to eliminate pathogens. Control of reproductive function takes place along the hypothalamic-pituitary-gonadal axis. GH secretagogues are a family of compounds capable of stimulating growth hormone secretion. Ghrelin is a 28 amino acid peptide with an integral role in the neuroendocrine control of GH release and the immune response in a variety of vertebrates. GHRP-6 (Growth Hormone Releasing Peptide-6) is one of the first synthetic agonists developed for the secretagogue receptor. These compounds mimic the effect of the endogenous ligand, ghrelin. In this study we evaluated the effect of administering GHRP-6 intraperitoneally on the expression of genes linked to the control of reproductive function and the immune system in these species. For the first time it is shown that the administration of GHRP-6 in juvenile tilapia (*Oreochromis niloticus) is capable of modulating the* mRNA expression levels of hormones linked to reproductive development: LH-β, FSH-β and PRL. This peptide also modulated the expression of the genes: IL-1β, MHC- IIb, MX, IgM, IgT and Granzyme. These results add new insights into the action of GHRP-6 in modulating the immune system in tilapia.

ORAL PRESENTATIONS

Title: Evading the antibodies – insights into antigenic variability in the myxozoan parasite *Sphaerospora molnari*.

Authors: Tomáš Korytář, Justin Tze Ho Chan, Jovana Majstorovic, Astrid Holzer

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Abstract:

With the emergence of adaptive immunity 400 mya ago, pathogens were faced with the ability of the host to recognize and remember any of the pathogens' surface antigens. To avoid antibody-mediated effector functions, pathogens across the tree of life developed various evasion strategies rendering the antibody responses ineffective. Using the model infection of common carp with myxozoan parasite *Sphaerospora molnari*, we aimed to shed more light on the protective capacity of S. molnari-specific antibodies and provide new information about the strategy of antigenic variability developed in one of the oldest clades of metazoan parasites.

To this end, we first investigated whether the *S. molnari*-specific antibodies conferred protection and elucidated the capacity of S. molnari to escape antibody-mediated elimination *in vitro* and *in vivo*. Furthermore, using the newly developed pan-S. molnari antibody, we studied the antigenic composition of independent parasite isolates and correlated it with the lytic potential of immune sera obtained during the experimental infection.

We demonstrated that, although the *S. molnari*-specific antibodies limit parasite proliferation through complement-mediated lysis, the immune sera do not prevent infection completely. Depending on the parasite's antigenic makeup, the parasite escapes the antibody-dependent elimination and establishes an infection even upon vaccination or secondary exposure, owing to the high variability of recognized surface antigens.

Collectively, the obtained results provide vital insights into the importance of antibody responses during myxozoan infections and point to the novel mechanism of antigenic variability in this ancient clade of cnidarian parasites.

ORAL PRESENTATIONS

Title: Immunomodulatory properties of Smolstatin, a cysteine protease inhibitor of the myxozoan parasite *Sphaerospora molnari*

Authors: Picard-Sánchez A., Bartošová-Sojková P., Chan J. T. H., Holzer A. S., Korytář T.

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Abstract:

Parasite cystatins are protease inhibitors; some interact with endogenous proteases of the parasite, while others interfere with host proteases and impact both the innate and adaptive immune responses. Recently, we described a novel cystatin of stefin type (Smolstatin) in the *Sphaerospora molnari*, the hemolytic myxozoan parasite of common carp *Cyprinus carpio*. Here we aimed to investigate its role in the host-parasite interaction, its immunomodulatory potential, and if we can immunize fish using Smolstatin.

To inquire whether the Smolastatin can impact the antigen uptake, degradation and presentation, we first investigated its impact on the phagocytosis and phagolysosome formation. Additionally, through gene expression profiling of the main inflammatory markers via qPCR, we investigated its immunomodulatory capacity in the induction of inflammation. Furthermore, the potential of Smolstatin as a vaccine candidate has been tested in parasite challenge.

The obtained results identified only a marginal effect of rSmolstatin on the phagocytic activity of carp leukocytes. Similarly, with the exception of *inos*, which underwent a several-fold upregulation, we observed mild increases in the expression of other tested pro- and anti-inflammatory cytokines. Finally, despite high levels of Smolstatin-specific antibodies induced by vaccination, we saw no differences in parasite burden in the vaccinated fish compared to the control group.

Collectively, the obtained data indicated only marginal immunomodulatory properties of this Smolstatin and future studies will be needed for delineating its role in host-parasite interactions.

Funding: Czech Science foundation 21-16565S, 19-28399X.

ORAL PRESENTATIONS

Title: Meta-analysis: a powerful tool in aquaculture

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Abstract:

The diversity of experiments in aquaculture in terms of density, stage, physical-chemical parameters makes conclusions and decision making very difficult. Recently, metanalysis is being used more frequently in aquaculture. The present work aims to show the meta-analysis as a tool for analysis of published data giving the scientific basis for decision-makers. The met-analyses in aquaculture have made it possible to identify the effect of probiotics on survival, conversion factor, and growth of shrimp farming. Similarly, the immune response was the subject of a meta-analysis with conclusions on the impact of culture conditions on the immune response. A meta-analysis is a powerful tool that allows the evaluation of scientific literature for decision-making, needing precaution due to the experimental design and the way the results are expressed.

ORAL PRESENTATIONS

Title: Novel insights into the role of IgD in teleost fish

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Abstract:
Immunoglobulin D (IgD) is an ancient antibody with dual membrane-bound and fluid-phase antigen receptor functions. However, to date, the precise role of IgD remains elusive both in mammals and in teleost fish. Recent work from our laboratory has demonstrated that $IgD^{+}IgM^{-}$ cells can be found in rainbow trout (*Oncorhynchus mykiss*), as reported in humans and other fish species. Interestingly, these $IgD^{+}IgM^{-}$ plasmablasts constitute a major lymphocyte population in some mucosal surfaces, including the gills and the gut mucosa, where they are specifically regulated in response to diverse stimuli. Remarkably, gut and gill IgD showed a tissue-specific V(D)J gene signature that reflected clonal expansion and mild SHM, which were absent in splenic IgD. Additionally, we found that, in the gut, secreted IgD bound to gut commensal bacteria, which in turn stimulated IgD gene transcription in gut B cells. These results suggests that, in rainbow trout, $IgD^{+}IgM^{-}$ plamablasts mount microbiota-regulated IgD responses that likely integrate those mediated by IgT and IgM. These IgD responses may promote gut homeostasis though a two-way interaction with the local microbiota. Altogether, our findings suggest that secreted IgD plays an evolutionary conserved role in mucosal homeostasis.

ORAL PRESENTATIONS

Title: Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) boosts antimicrobial defenses in teleost fish: development of an oral formulation.

Authors: Janet Velázquez[1], Alianet Rodríguez[1], Geysi Pérez, Shawna L. Semple[1], Tania Rodríguez-Ramos[2], Patricia Díaz-Rosales[3], María del Camino Ordás, Juana María Lugo[1], Carolina Tafalla[3], Brian Dixon[2], Fidel Herrera[1], [4]Paola Orellana, [4]Joceline Ruiz[4], Matías Vega[5,6], Alex Romero[5,6,] Néstor Santos[1], Gerardo Ramsés[1], Antonio Morales[1], Osmany Rodrigo[1], Patricio Dantagnan[7], Mario Pablo Estrada[1] Yamila Carpio[1]

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Abstract:

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that belongs to the secretin/glucagon/GHRH/VIP superfamily. Some of these molecules have antimicrobial activity and they are capable of stimulating the immune system. The present work studied the immunomodulatory, antibacterial and antiviral activity of PACAP-38 from African catfish *Clarias gariepinus* in this specie and in rainbow trout *Oncorhynchus mykiss*. With this knowledge in advance, we evaluated the physiological effects of a new feed formulation containing *C. gariepinus* synthetic PACAP-38 administered to rainbow trout fingerlings. A new oiled formulation containing the peptide was developed and two diets with different PACAP concentrations were prepared. After two months of administration, dietary supplementation with PACAP markedly improved the PUFA content in muscle, increased the total number of caliciform cells within the intestinal villi and modulated the transcription of some of the studied cytokines in head kidney. This study suggests new potential outcomes in further advancing of the use of small peptides as feed additives and formulations to improve the quality of culture fish fingerlings.

ORAL PRESENTATIONS

Title: Potential effect of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP-38) with its variants against *Flavobacterium psychrophilum, Flavobacterium columnare, and Aeromonas salmonicida.*

Authors: Lowia Al-Hussinee[1], Tania Rodríguez Cornejo[1], Tania Rodríguez-Ramos[1], Janet Velázquez Pérez[2], Yamila Carpio[2], Mario P. Estrada[2], and Brian Dixon[1]

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Abstract:
Upon the discovery of Pituitary adenylate Adenylate Cyclase-Activating Polypeptide (PACAP) from the ovine nervous system, its multiple functions have been defined, including in the cardiovascular, nervous, muscular, but mainly immune systems. PACAP exerts a potential role as an anti-inflammatory and immunomodulatory factor against several bacterial pathogens in mammals and teleosts. In this study, we examined the effect of PACAP-38 (labelled PACAP-1), as well as three other PACAP-38 variants (labelled PACAP 2-4) plus one variant (labelled PACAP-5) that was scrambled to produce a negative control, against *Flavobacterium psychrophilum, Flavobacterium columnare, and Aeromonas salmonicida.* Inhibition of bacterial growth using minimum inhibition concentration (MIC) technique and immune stimulation of cytokines in PACAP treated fish macrophage & brain cell lines post *in vitro* with/out bacterial infection were examined. The MIC study indicates that PACAP- 3 and 4 inhibit ~ 100% of bacterial growth of *Aeromonas salmonicida, Flavobacterium psychrophilum* and *Flavobacterium columnare*, at concentrations as low as 5 µM, and 35 µM, respectively. There is also evidence of upregulation of TNF-α, IL-1β, IL-6, & IL10 in brain and macrophage cell lines treated with PACAP. Further to that our examination of macrophage cell lines shows a significant upregulation of IFN-g & IL-1β in cells treated with PACAP after infection with *Flavobacterium columnare,* while TNF-α & IL-10 cytokine stimulation was observed in the brain cell line. Our initial study of PACAP variant cytotoxicity on red blood cells of rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*) indicates minimum effects of PACAP 1-3, and 5 on blood hemolysis. This study gives us key details needed for the use of PACAP variants in aquaculture as an antibacterial therapeutic agent.

ORAL PRESENTATIONS

Title: RNAseq data analysis of Oreochromis niloticus tilapias treated with Growth Hormone Releasing Peptide-6

Authors: Frank Torres, Daniela Felipe Moro, Adrián Rodríguez, Rebeca Martínez, Mario Pablo Estrada, Ricardo Bringas

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Abstract:

We present the bioinformatics data analysis of an experiment aiming to evaluate the effect of peptide GHRP-6 treatment in *Oreochromis niloticus* tilapias. The experiment collected samples from 4 different organs (gill, spleen, head kidney and liver) and two different doses of treatment. We performed differential expression analysis in the eight conditions. The number of differentially espressed genes (DEG) was very diverse between conditions. While samples from liver and head kidney showed more than 200 DEGs, samples from gill and spleen had less 20 DEGs each. For the low dose condition in the spleen non DEGs were found. In general high and low doses showed similar results. HK and Liver samples showed similar number of DEGs in both doses, but significantly more down- than up-regulated genes Functional enrichment analysis was performed for each sets of up- and down-regulated genes in each condition. Several samples showed up-regulation of genes related to the immune system. Additionally, was performed a sequence analysis of Up-regulated genes promotors with the aim to identify transcription factors regulatory motifs implicated in the differential expression induced by the GHRP-6 treatment. Both enrichment and sequence analysis evidenced the activation of immune response related genes.

ORAL PRESENTATIONS

Title: Simultaneous analysis of 12 distinct populations of leukocytes following pathogenic challenge in rainbow trout (*Oncorhynchus mykiss*).

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Abstract:
Knowledge on cellular immune responses in fish species has been hindered by the absence of reliable monoclonal antibodies recognizing different fish leukocytes populations. In the past we have produced mAbs to rainbow trout IgT and CD4, which have enabled the characterization of IgT^+ B cell and $CD4^+$ T cell subsets respectively. More recently we have developed mAbs to gamma delta T cells (gd T cells) and natural killer cells (NK cells) which have greatly expanded our capabilities to analyze and understand both innate and adaptive cellular immune responses in fish. The mAbs to trout gd T cells identified three main subsets of gd T cells, including $CD4\text{-}2^+$ gd^+, $CD8^+$ gd^+, $CD4^-CD8^-$ gd^+ T cells. Similar to the situation in mammals, trout gd T cells were prevalently found in mucosal surfaces, especially those of the gut and gill. Trout NK cells were mainly localized in the head kidney although they could also be detected in small numbers in other lymphoid tissues. The development of these new mAbs along with the ones previously developed by our and other labs, enables the concurrent identification of at least 12 different subsets of leukocytes in trout, including $CD4\text{-}2^+$ gd^+, $CD8^+$ gd^+ and $CD4^-CD8^-$ gd^+ T cells, $CD4\text{-}1^+$, $CD4\text{-}2^+$ and $CD4\text{-}1^+CD4\text{-}2^+$ T cells, $CD8^+$ and $CD4^+CD8^+$ T cells, IgT^+ and IgM^+ B cells, NK cells and macrophages. These subsets can be further dissected into additional subsets, based on the staining intensity provided by each mAb as well as the analysis by flow of several cell properties (i.e., size, granularity, activated or resting state). As an example, IgT^+ B cells are comprised of subpopulations of IgT^{low} or IgT^{hi} large (i.e., plasmablast-like cells) and small (i.e., lymphocytes) resting or proliferating cell subsets. The ability to concurrently analyze all of the aforementioned leukocyte subsets offers unprecedented opportunities for the evaluation of the cellular components of the innate and adaptive immune response upon challenge of fish with a pathogen or treatment of fish with a vaccine, immunostimulant or probiotic. We developed a multicolor flow cytometry methodology that enabled the simultaneous detection of resting or proliferating leukocyte subsets. This multicolor flow cytometry approach was then used to analyze the kinetics of leukocyte responses in systemic and mucosal lymphoid organs upon challenge of fish with sublethal doses of Ich (*Ichthyophthirius multifiliis*), *Yersinia ruckeri* or *Flavobacterium columnare*. Results obtained from the analysis of the different leukocyte populations have offered previously unrecognized clues into the mechanisms by which innate and adaptive immune responses are induced in systemic and mucosal lymphoid organs upon pathogenic challenge. We predict that the use of this multiflow assay will not only shed light into the cellular immune responses against pathogens induced by teleosts, but will provide also the knowledge to understand how immune responses are induced upon treatment of fish with novel vaccines, immunostimulants and probiotics.

ORAL PRESENTATIONS

Title: Study of the role of Immunoglobulin T in the humoral immune response in Nile tilapia (*Oreochromis niloticus*)

Authors: Janet Velázquez[1], Alianet Rodríguez[1], Lynn Cruz[1], Hasel Aragón[2], Maylin Pérez-Bernal[3], Onel Valdivia[3], Arlette Haidar[1], Fidel Herrera[1], Osmany González[1], Antonio Morales[1], Lisbet Ulloa[3], Reinaldo Blanco[4], Joel Pérez[4], Dayamí Dorta[4], Yaramis Luna[4], Marcos González[2], Rodolfo Valdés[2], Hilda Elsa Garay[5], David Diago Abreu[5], Yassel Ramos[6], Vladimir Besada[6], Ania Cabrales[6], Yeosvany Cabrera[3], Mario Pablo Estrada[1], Yamila Carpio[1]

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Abstract:

Immunoglobulin molecules play an important role in the immune defense system in all jawed vertebrates. Nile tilapia (*Oreochromis niloticus*) is a freshwater fish extensively cultivated worldwide and constitutes one of the model species for the study of fish immunology. The presence of the immunoglobulin M gene in this species is well documented, as well as its major role in systemic immunity. To date, the IgT gene from *O. niloticus* has not been identified and, therefore, no information is available on the role of this immunoglobulin isotype in the immune response in tilapia. In the present work, novel secreted and membrane immunoglobulin T isotypes were isolated from tilapia head kidney lymphocytes. The transcriptional profiles of IgT and IgM were analyzed by quantitative PCR in larval developmental stage and in different tissues of healthy or lipopolysaccharide/*Edwardsiella tarda*-challenged tilapia adults. Our results suggest a potential involvement of this new Ig in mucosal immunity in tilapia. Monoclonal antibodies are very advantageous molecular tools for studying teleost immune system. In the present study, we produced and characterized monoclonal antibodies against tilapia IgT and IgM heavy chain using a peptide-based strategy. Specificity of the mAbs was confirmed by ELISA, Western blotting and Flow cytometry. This tool allowed us to study for the first time the stimulation of mucosal immunity after Pituitary Adenylate Cyclase Activating Polypeptide administration, and the effect of TT-P0 *Ls* vaccine candidate against sea lice on the proliferation of IgM^+ and IgT^+ B cell populations in tilapia and on mucosal immune response. Current research provides an important immunological tool for studying and deepens our understanding on the biological function and structural characteristics of tilapia humoral immune response.

ORAL PRESENTATIONS

Title: The sea lice microbiota: a potential threat for salmon aquaculture

Authors: Diego Valenzuela-Miranda*, Ana Teresa Gonçalves, Valentina Valenzuela-Muñoz, Gustavo Nuñez-Acuña & Cristian Gallardo-Escárate

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Abstract:

The sea louse (*Caligus rogercresseyi*) is a marine ectoparasite that has become one of the main constrains for the sustainable development of Chilean salmon aquaculture. Besides the well-known deleterious effects of sea lice in salmon farming, novel evidence suggests the presence of a large and diverse microbiota in the parasite. However, the biological roles in the parasite development and the potential threats for salmon farming remains unexplored. In this scenario the present work was aimed to (i) characterize sea lice microbiota from distant populations, (ii) to predict biological roles of the microbial community in the development of sea lice, and to, (iii) to identify bacterial pathogens that could potentially impact salmon aquaculture. To do this, chromosome proximity ligation (Hi-C) coupled with long-read sequencing were used for the genomic reconstruction of the *C. rogercresseyi* microbiota, while nanopore sequencing of the full 16S rRNA gene was used for microbial profiling at specie level. Through Hi-C we were able to assemble and characterize 413 bacterial genome clusters, including six bacterial genomes with more than 80% of completeness. The most represented bacterial genome belonged to the fish pathogen *Tenacibacullum ovolyticum* (97.87% completeness), followed by *Dokdonia sp.* (96.71% completeness). This completeness allowed identifying 21 virulence factors (VF) within the *T. ovolyticum* genome and four antibiotic resistance genes (ARG). Notably, genomic pathway reconstruction analysis suggests putative metabolic complementation mechanisms between *C. rogercresseyi* and its associated microbiota. Regarding possible bacterial pathogens in sea lice microbiota, a total of 30 potential fish bacterial pathogens species were identified. Notably, fourteen Vibrio spp. were predominantly found in the Los Lagos region, while six Tenacibaculum spp. were more equally distributed among the sites. A core of five fish pathogens was observed in all farming zones, including *Aliivibrio wodanis*, *T. dicentrarchi*, *T. ovolyticum*, *T. soleae*, and *V. splendidus* (see figure). Overall, our results evidence that sea lice microbiota might fulfill key metabolic roles in the parasite's development. At the same time, potential threats for salmon farming were found within the microbiota, including fish bacterial pathogens, virulence factors and antibiotic resistance genes.

Funding: ANID-Chile through the Postdoctoral grant FONDECYT (#3200600), and FONDAP (#15110027).

ORAL PRESENTATIONS

Title: Transcriptome modulation of *Salmo salar* immunized with *Caligus rogercresseyi* vaccine prototype: a host-parasite interaction.

Authors: Antonio Casuso[*1], Valentina Valenzuela-Muñoz[1], Cristian Gallardo-Escárate[1]

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Abstract:

Caligus rogercresseyi is an ectoparasitic that produces the greatest economic losses in the salmon industry. Therein, vaccine-based control strategies for this sea louse have long been desired. The genome and the transcriptome data reported for *C. rogercresseyi*, allow the identification of putative antigens using the reverse vaccinology approach. This study aimed to evaluate the efficacy of vaccine prototypes during the sea lice infestation and their effects in host-parasite transcriptome modulation. Atlantic salmon were immunized with recombinant proteins peritrophin, and cathepsin identified from sea louse genome. Four experimental group were vaccinated with different prototypes peritrophin, cathepsin, peritrophin/cathepsin (P/C) combination and PBS as control. Follow 400 UTAs, vaccinated salmons were infested with 35 copepodid per fish. Sea lice attachment were evaluated at 7 and 25 days post infestation (dpi). Samples of head kidney and skin tissues, and *C. rogercresseyi* female were taken for mRNA Illumina sequencing. RNA-seq analysis were performed. Moreover, for contigs differently express GO and KEEG pathway analysis were performed. Fish vaccinated with cathepsin, and P/C showed 57% efficacy, reducing adult lice bunder. Transcriptome analysis indicated a vaccine-dependent gene modulation, both at 7 and 25 dpi. Notably, at 7 dpi fish vaccinated with P/C and cathepsin showed an upregulation of genes associated whit metal ion binding, molecular processes energy production comparing with the control group. While at 25 dpi for Atlantic salmon and sea lice, genes associated with ATP binding, iron ion binding and zinc ion binding were strongly upregulated. The results suggested that the vaccine prototypes stimulate energetic metabolism in salmon at 7 dpi. In addition, competition for metal ions between the host and the parasite in the infestation was evidenced. Finally, this study uncovered the molecular responses produced during host-parasite interaction in vaccinated fish and provide a new strategy for sea lice control in the salmon industry.

Funding: This study was funded by FONDAP grant #15110027 and ANID-PCHA/Doctorado Nacional (Grant 2018-21180084).

ORAL PRESENTATIONS

Title: Whole-genome expression approach for transcriptome analysis to understand Atlantic salmon seawater adaptation

Authors: Valentina Valenzuela-Muñoz[*1,2], Cristian Gallardo-Escárate[1,2], Bárbara P. Benavente[1,2], Diego Valenzuela-Miranda[1,2], Gustavo Núñez-Acuña[1,2]

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Abstract:

The available high resolution of genome information and transcriptomes data allows the understanding of complex biological processes. However, the analysis of complex experimental designs involving different tissues, times-points or environment represents the main obstacle. This study proposes a novel approach to analyze complex data sets combining coding and non-coding RNAs at the chromosome-level genome. Therein, Atlantic salmon smolts were transferred to SW under two strategies. (i) Fish group exposed to gradual salinity changes (GSC), and (ii) exposed to a salinity shock (SS). Gills, intestine, and head kidney samples were used for total RNA extraction, followed by mRNAs and small RNAs Illumina sequencing. Through a whole-genome transcriptomic approach, different expression patterns among the tissues and treatments were observed. A mRNAs and miRNAs correlations expression were observed at chromosome levels. Chromosome regions highly expressed between experimental conditions included a high abundance of transposable elements. In addition, differential expression analysis showed a higher number of transcripts modulated in response to SS in gills and head kidney. miRNAs expression analysis suggested a low number of miRNAs involved in the smoltification process. However, the target analysis of these miRNAs showed a regulatory role in growth, stress response, and immunity. This study is the first evidencing the interplaying among the mRNAs/ miRNAs and the structural relationship at genome level during Atlantic salmon smoltification.

Funding: ANID-Chile funded this study through the Postdoctoral grant FONDECYT (3190320), grants FONDAP (15110027) and FONDECYT (1210852).

ORAL PRESENTATIONS

Title: Whole-genome resequencing of the sea lice *Caligus rogercresseyi* reveals novel genomic markers associated with pharmacological resistance

Authors: Gustavo Núñez-Acuña*, Constanza Sáez-Vera, Valentina Valenzuela-Muñoz, Diego Valenzuela-Miranda and Cristian Gallardo-Escárate

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Abstract:

The sea louse *Caligus rogercresseyi* is a marine ectoparasite copepod species that impacts the salmon production in Chile, causing losses of more than 400 million dollars per year. This pathogen is mainly controlled by immersion baths with delousing drugs, which are directly applied in salmon cages covered by skirts or tarpaulins. The emergence of resistance has been suggested as the main cause of low efficacy of delousing drugs treatments against sea lice infection. Pharmacogenomics approaches are now possible to conduct in *C. rogercresseyi*, having the draft of the full genome as a reference to infer these duplications as copy number variants (CNVs) in different resistant or susceptible strains. This study aimed to evaluate the presence of gene duplications, or gene-clusters duplications, related to genes with functions in delousing drug response, and its association with resistant phenotypes to azamethiphos in *C. rogercresseyi*. The full genome of *C. rogercresseyi* was used as a reference to conduct whole-genome resequencing for known sea louse strains with divergent resistance to azamethiphos drug. Then, gene-clusters duplications in the novel specific whole-genome sequences for resistant and susceptible strains were identified and associated with resistant sea lice. Copy number variants (CNVs) in functional genes were identified with differential p-value among resistant and susceptible strains, but most of the differential variants were found in transposable elements. Duplicated regions also implied expression changes in these strains. The potential impact of this study for salmon aquaculture is the definition of novel resistant traits in families or populations of sea lice, and the identification of novel molecular markers based on CNVs, supporting the creation of monitoring programs for *C. rogercresseyi* resistance to delousing drugs.

Funding: ANID-Chile through the grant FONDECYT (#11200813 and # 1210852), and FONDAP (#15110027).

POSTERS

Notice:
P# stands for the number of the panel where the poster must be exhibited.
Abstracts are indexed by title in alphabetical order.

P01 ACUABIO V: Organization of production. Authors: Laura González González

P02 Analysis of RNAseq data of *Oreochromis niloticus* tilapias treated with GHRP-6 peptide. Frank Torres et al.

P03 Antimicrobial peptide Oreochromicin-2: Pre-formulation stability studies and new insights in the antimicrobial action against *Pseudomonas aeruginosa*. Liliana Basabe-Tuero et al.

P04 Behavior of infectious and contagious diseases in shrimp farming (*L. vannamei*) in Cuba from 2019-2021. Maylee Pozo Escobar et al.

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P06 Characterization of polyclonal antibodies generated against interferon gamma in Nile tilapia (*Oreochromis niloticus*) by western blot, ELISA and flow cytometry. Lynn Cruz et al.

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P19 Producción de microalgas para la alimentación de estadios tempranos del camarón blanco *Penaeus vannamei* en el centro de producción de larvas YAGUACAM. Yaniel Batista Nodal, MSc. Zurisleidy González Clak

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POSTERS

P01 Title: ACUABIO V: Organization of production

Authors: Laura González González[1]; Yandiesky Lowery Veitía[2]; Norelbys Albelo Rondón[2], Carmen Chuay Silva[1]

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Abstract:
Acuabio V® is a nutritional supplement indicated to increase the size, weight and survival of aquatic organisms during the first stages of life subjected to culture. The aim of this work was to obtain the Sanitary Registration and the availability of the product for field studies, therefore it was required to comply with the Good Production Practices in the different facilities (National Biopreparades Center, BioCen and National Center for Agricultural Health, CENSA) proceeding to the final filling at the Center for Genetic Engineering and Biotechnology, (CIGB). This work established the production process organized as to obtain the finished product Acuabio V®, its productive and documentary flow in each possible scenario, as well as the traceability and quality. As a result, the gaps of the stated processes were identified and the delivery was guaranteed for continuing of studies and obtaining the Sanitary Registration of the Product

POSTERS

P02 Title: Analysis of RNAseq data of *Oreochromis niloticus* tilapias treated with GHRP-6 peptide.

Authors: Frank Torres, Daniela Felipe Moro, Adrián Rodríguez, Rebeca Martínez, Mario Pablo Estrada, Ricardo Bringas

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Abstract:

We present the bioinformatics data analysis of an experiment aiming to evaluate the effect of peptide GHRP-6 treatment in *Oreochromis niloticus* tilapias. The experiment collected samples from 4 different organs (gill, spleen, head kidney and liver) and two different doses of treatment. We performed differential expression analysis in the eight conditions. The number of differentially espressed genes (DEG) was very diverse between conditions. While samples from liver and head kidney showed more than 200 DEGs, samples from gill and spleen had less 20 DEGs each. For the low dose condition in the spleen non DEGs were found. In general high and low doses showed similar results. HK and Liver samples showed similar number of DEGs in both doses, but significantly more down- than up-regulated genes Functional enrichment analysis was performed for each sets of up- and down-regulated genes in each condition. Several samples showed up-regulation of genes related to the immune system. Additionally, was performed a sequence analysis of Up-regulated genes promotors with the aim to identify transcription factors regulatory motifs implicated in the differential expression induced by the GHRP-6 treatment. Both enrichment and sequence analysis evidenced the activation of immune response related genes.

POSTERS

P03 Title: Antimicrobial peptide Oreochromicin-2: Pre-formulation stability studies and new insights in the antimicrobial action against *Pseudomonas aeruginosa*.

Authors: Liliana Basabe-Tuero[1]*, Raymersy Aldana Wilson[2]*, Liany Coto Guerra[1], Soraya Piloto Díaz[3], Hilda Garay Pérez[4], Ana Aguilera Barreto[2], Mario Pablo Estrada García[5], Rebeca Martínez Rodríguez[1].

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Abstract:

Antimicrobial Peptides (AMPs) are a new alternative for therapeutic agents to face the rapid increase of antibiotic-resistant pathogens which posed a great threat to animal and human health and life. Oreochromicin-2 (Oreoch-2), an AMP isolated from *Oreochromis niloticus*, has shown to be active against a broad spectrum of bacteria, including *Pseudomonas aeruginosa*, a well-known pathogen responsible for considerable economic losses in aquaculture. However, the proteolytic degradation of peptides and the lacking of knowledge about their modes of action are often major restrictions to develop them as peptide-based drugs. We studied the effect of pH and different buffer compositions on peptide stability and some insights in the antimicrobial activity and possible action mode against this gram-negative bacterium were also explored. Oreoch-2 stability was evaluated at pH ranging from 3-8 and different buffer compositions at 4°C and 60°C. The peptide is stable at pH 5,6 in acetic acid/sodium acetate buffer at the two temperatures studied. The time-kill kinetics of *P. aeruginosa* demonstrated this AMP exhibit strong bactericidal effect against this bacterium, with an impressive bactericidal effect at concentrations below 3µM in a short assay time. Besides, it was found that Oreoch-2 interacts with *P. aeruginosa* genomic DNA in a dose- and time-dependent manner. This finding suggests the mechanism of action could involve DNA interaction. Results support Oreochromicin-2 may represent a promissory alternative to antimicrobials currently available for combating infectious diseases in aquaculture.

POSTERS

P04 Title: Behavior of infectious and contagious diseases in shrimp farming (*L. vannamei*) in Cuba from 2019-2021

Authors: Lic. Maylee Pozo Escobar, Lic. Lirialis García Mesa, Lic. Darleny Pérez Quesada, Lic. Eolian Rodríguez Vara, MsC. Fernando Lucas Prats, Dr.C Manuel Rubio Limonta, Lic. Sheyla Ponte Betancourt, Lic. Suyin Matos Pons, DraC. Raquel Silveira Coffigny

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Abstract:

Infectious diseases in shrimp farming are an important factor for success in farming. Its presence in animals can cause severe economic losses. To avoid this problem in Cuba, periodic sampling has been carried out in shrimp farms throughout the country to identify health problems in these aquatic animals. That is why the objective of this work is to determine the incidence of diseases in shrimp (*L. vannamei*) in culture in the period 2019-2021. The analyzes performed include the description of clinical signs, bacteriological analyzes of the hepatopancreas, detection of *Hepatobacter penei* (NHP), White Spot Syndrome virus (WSSV), Yellow Head Virus (YHV), myonecrosis virus infection (IMNV), Taura Syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), acute hepatopancreatic necrosis disease (AHPND) and epicommensals. Histopathological analyzes of hepatopancreas, gills and muscle were also performed. The main clinical signs observed in the period coincide with the reddish coloration of the pleopods and uropods, and a reddish intestine; soft exoskeleton, light melanosis in the form of macules, friable and whitish hepatopancreas. The results show that there is a high incidence of epicommensals, during all years with values above 50%, followed by NHP and, to a lesser extent, vibriosis infection. No viral illnesses were reported in the analyzed period. The reported epicommensals correspond to the genus *Epistylis sp*. The most frequently isolated bacterial species were *Vibrio cincinnatiensis*, *Vibrio orientalis*, *Vibrio mimicus*, and *Vibrio parahaemolyticus*.

POSTERS

P05 Title: Characterization of the identified biohazards from fingerlings of *Clarias gariepinus* farmed in Cuba

Authors: Lic. Eolian M. Rodríguez Vara[1]*, MSc. Fernando Lucas Prats León[1], Téc. Mercedes Martínez Pérez[1], DraC. Raquel Silveira Coffigny[1], DraC. Ernestina Solórzano Álvarez[2]

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Abstract:

In aquaculture, biosafety proposes to apply adequate measures to reduce the probability of biological agent would spread to an individual, a population or an ecosystem and to moderate its negative effects. Within the culture of aquatic animals in Cuba, the production of *Clarias gariepinus* stands out. The knowledge about the parasitic trichodinids that affect this commercial species in culture conditions and the risks their presence implies, is an important work objective for the industry. In the present work, the main species of parasitic trichodinids that constitute biological hazards for the fingerling stage of *C. gariepinus* cultivation in Cuba were characterized. The study was carried out at the La Juventud Fingerling Center, Pinar del Río, where periodic samplings were taken between the years 2017-2019 to collect the trichodinids from living fingerlings. The identified biological hazards were the three species of the genus *Trichodina*: *T. acuta* Lom, 1961; *T. heterodentata* Duncan, 1977 and *T. merciae* Prats and Martínez, 2017. These species cause tricodiniasis in clarias fingerlings, a disease that causes irritation of the epithelial layer of the skin and fusion of the gill filaments. Those damages affects the optimal respiratory and excretory capacities of the gills, as well as the homeostatic and osmoregulatory functions of the skin. Several infestations with these pathogens cause deep ulcerations on the skin surface that act as entry sites for secondary bacterial or fungal infections. The present identification and characterization of the trichodinids that affect the culture of *C. gariepinus* during their fingerling stage, constitutes the first step to develop a risk analysis that guarantees the right biosafety management of aquaculture stations where this fish species is farmed

POSTERS

P06 Title: Characterization of polyclonal antibodies generated against interferon gamma in Nile tilapia (*Oreochromis niloticus)* by western blot, ELISA and flow cytometry

Authors: Lynn Cruz[1*], Janet Velázquez[1], Geysi Pérez[1] Antonio Morales[1], Osmany González[1], Fidel Herrera[1], Mario Pablo Estrada[1], Yamila Carpio[1]

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Abstract:

The validation of a polyclonal antibody is a process that is carried out using analytical techniques, to demonstrate its sensitivity, specificity and reproducibility in the context in which they are going to be used. In previous work, the Nile tilapia IFNγ (*Oreochromis niloticus*) was isolated and cloned for the first time. Also, polyclonal sera were obtained in mice and rabbits using the recombinant protein or a synthetic immunogenic peptide derived from tilapia IFNγ as immunogens. In the current study, the polyclonal antisera from both animal species were purified by affinity chromatography in denaturing conditions and size exclusion chromatography. The specificity and utility of purified antibodies was evaluated by Western blot and ELISA, obtaining the best results with the polyclonal antibody obtained in rabbits using the peptide P65. Flow cytometry to study spleen, head kidney and PBL IFN+ lymphocyte populations was performed. Further, an *in vivo* assay was done by injecting a sea lice vaccine candidate and evaluates its effect over IFN production. Samples of spleen, head kidney and peripheral blood were taken at 3 and 7 days post-booster and the percentages of IFNγ+ cells were determined by flow cytometry after in vitro stimulation with the antigen at 72 hours post-stimulation. This pAbs allowed us to determine for the first time the percentage of IFNγ+ cells in basal conditions and stimulation in Nile tilapia

POSTERS

P07 Title: Current situation of the industrial property portfolio associated with aquatic biotechnology at Center for Genetic Engineering and Biotechnology, Cuba

Authors: Sonia González[1], Yamila Carpio[2], Rebeca Martínez[2], Liliana Basabe[2], Alina Rodríguez[2], Mariela Vázquez[1], Raimundo Ubieta[1], Mario Pablo Estrada[2]

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Abstract:

Aquaculture is a fastest growing food-producing sector. For decades, researchers at the Center for Genetic Engineering and Biotechnology (CIGB) have been involved in the development of biotech products that impact in aquaculture, and their innovative results were protected by patents. PACAP, is a well-known pleiotropic neuropeptide relevant to various biological processes in mammals. However, the aquaculture biotechnology group of CIGB was the first to identify its role as a growth promoter in fish and crustaceans. Later, said researchers were also able to assign a role to this neuropeptide as an antiviral molecule. In addition, for the first time, they demonstrated that PACAP enhances the specific immune response to a co-administered antigen in several animal species. All three inventions were filed internationally, and are the origin of three patent families including, at present, more than 50 patent documents, valid in most of the regions of the world. Similarly, the hexapeptide GHRP-6 has been in the target of aquatic biotechnology research at CIGB, as a growth stimulator and as a vaccine adjuvant. Said intense research gave place to two patent families. In another attempt to benefit aquatic organism's health, three antimicrobial peptides derived from tilapia were identified and protected in a patent application filed in 2011. Once the patent application was filed internationally, it originated patents already granted in several jurisdictions. Moreover, recombinant, synthetic, or conjugated antigens for the prevention of sea lice infestations were also protected in patent applications filed in Cuba and abroad. The relevant antigens are the subject matter of two patent families, valid in most of the regions of the world. At present, there are more than 100 valid patents owned by CIGB that are related to aquatic biotechnology, and it is an important part of the industrial property portfolio of our center

POSTERS

P08 Title: Determination of the microbial load in batches of a vaccine candidate against ectoparasites at the CIGB Camagüey

Authors: Frank Álamo Hernández[1], Niuvis Montoya Echavarría[1], Yunier Paneque[1], Yanelkis Quesada Sarduy[1], Diasmarys Salinas[1], Ruthdali Segura[1], Alain Moreira[1], Mirlleys Peláez[1], Odisa Esquivel[1], Liyoesmin Salinas[1], Yamila Carpio[2], Alianet Rodríguez[2], Irian Mendoza Rodriguez[1], Jocelyne García Souto[1], William Pena Guimarais[1], Rosa Basulto Morales[1] and Nemecio González[1]

Affiliations: [1] Center for Genetic Engineering and Biotechnology, Camagüey, Cuba. [2] Center for Genetic Engineering and Biotechnology, Havana, Cuba.

Abstract:

Bioburden is a measure of microbial contamination or microbial load, the number of microorganisms that contaminate an object or liquid sample. In the pharmaceutical industry the microbial limit is carried out to evaluate the microbial levels between samples of intermediate and/or sterile products. The objective of the work was to carry out the bioburden test on the samples of the production process of the vaccine candidate against ectoparasites that affect aquaculture through the membrane filtration method. The results were expressed using the method of total count of microorganisms/amount of sample. Samples from lots 68N.2107, 68N.2108, 68N.2109 and 68N.2201 were processed. The process control samples of the vaccine candidate did not meet the established limits, being higher than <5 CFU/ 50 mL of sample. While the Active Ingredient samples did meet the limit established for the test, contaminants were not recovered on the membrane. The efficacy of the bioburden technique was verified to determine the amount of contaminants present in the non-sterile vaccine candidate product against ectoparasites that affect aquaculture.

POSTERS

P09 Title: Digestive lipolytic activity in the spiny lobster *Panulirus argus* and *in vitro* digestibility of lipid sources for lobster feeds

Authors: Leandro Rodriguez-Viera[1*], Erick Perera[2], Isabel M. Agredano Pila[3], Francisco J. Moyano[3], Juan M. Mancera[4], Manuel Díaz[3]

Affiliation: [1] Center for Marine Research, University of Havana, Havana, Havana, Cuba, [2] Institute of Marine Sciences of Andalusia (ICMAN), Spanish National Research Council (CSIC), 11519, Puerto Real, Cádiz, Spain, [3] Department of Biology and Geology, University of Almeria, 04120 Almeria, Spain, [4]Faculty of Marine and Environmental Sciences, Campus de Excelencia Internacional del Mar (CEIMAR), University of Cadiz, Puerto Real, Cadiz, Spain

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Abstract:

Spiny lobster aquaculture has increased during the past decades, attended to their high demand and commercial value. However, the absence of cost-effective and nutritionally adequate formulated feeds remains as one of the drawbacks for the sustainable expansion of this activity. Despite lipids are important nutrients and energy sources for these crustaceans, lipid digestion remains poorly understood. In this study, the pH-Stat method was used for studying in vitro the lipolytic activity in the digestive gland of the spiny lobster *Panulirus argus* and the digestibility of 18 oils from animal, plant and microalgae origin. We complemented these analyses by evaluating the effects of several emulsifiers (non-ionic surfactants, protein emulsifiers, and phospholipids) on lipid digestion, and by making a partial characterization of enzymes with lipolytic activity. Results demonstrate for the first time the presence of true digestive lipases in spiny lobsters, and also that *P. argus* has the ability to efficiently hydrolysed both animal and vegetable oils. Fish oils are highly digestible, but also rapeseed and algae oils. Also, results indicate that hydrolyzed soy lecithin has the greatest potential to be used as an emulsifier ingredient in lobsters feeds. This study increases our understanding on lipid digestion in lobsters and may assist in selecting appropriated oils and emulsifiers for *P. argus* feeds.

POSTERS

P10 Title: Effect of GHRP-6 peptide on mucosal IgT levels in skin and gills of tilapia (*Oreochromis niloticus*)

Authors: Amanda Comellas-Quicute[1*], Adrián Rodriguez-Gabilondo[1], Yaira Rojas-Castro[1], Liz Hernández[1], Janet Velazquez[2], Yamila Carpio[2], Antonio Morales[1], Fidel Herrera[1], Osmany Rodrigo[1], Mario Pablo Estrada[3], Rebeca Martínez[1]

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Abstract:

Aquaculture is the fastest growing food production sector in the world. The intensification of fish farming in the face of the growing demand for food caused by the increase in the world population has caused an increase in infectious diseases in these organisms, and consequently, a decrease in productive yields. In this scenario, the use of molecules such as immunostimulants is attractive. The innate immune system of fish is considered the first line of defense against a wide spectrum of pathogens. Immunoglobulins play an important role in the immune defense system in all vertebrates, protecting the organism from a wide variety of pathogens. In our laboratory we use tilapia as an animal model to study the effectiveness of the products we develop, so the ability to measure the humoral immune response greatly facilitates the evaluation and understanding of the immune response in tilapia. GH secretagogues are a family of compounds capable of stimulating growth hormone secretion. Ghrelin is a 28 amino acid peptide with an integral role in the neuroendocrine control of GH release and the immune response in a variety of vertebrates. GHRP-6 (Growth Hormone Releasing Peptide-6) is one of the first synthetic agonists developed for the secretagogue receptor. These compounds mimic the effect of the endogenous ligand, ghrelin. In this study we evaluated the effect of administering GHRP-6 intraperitoneally on mucosal IgT levels in the skin and gills of juvenile tilapia (*Oreochromis niloticus*). For the first time, the action of GHRP-6 in the stimulation of IgT levels in tilapia mucus is demonstrated..

POSTERS

P11 Title: Exploring pathogenicity attenuation of the intracellular bacterium *Piscirickettsia salmonis* through small non-coding RNA

Authors: Yeny Leal, Valentina Valenzuela-Muñoz, Cristian Gallardo-Escárate

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Abstract:
The intracellular bacterium *Piscirickettsia salmonis* is the responsible pathogen of salmonid rickettsial septicemia (SRS) and can infect multiple tissues in its host. Advances in high-throughput sequencing technologies allow a better understanding of the transcriptomic responses of organisms in several biological scenarios, such as pathogen-host interaction. In this sense, miRNAs play an essential role in the transcriptomic response of *Salmo salar* during infection with *P. salmonis*, promoting a change in the diversity of miRNA families. miRNAs co-modulate the transcriptional activity of their target genes, suggesting a putative function of non-coding RNAs in the immune response of salmon infected with an intracellular pathogen. This study aimed to identify candidates for small non-coding RNA (sRNA) involved in the pathogenesis process of *P. salmonis* during infection in *S. salar* and validate at a functional level the genomic modulation of these sRNA at in *vitro* model of *P. salmonis* infection. Transcriptome of experimental infections with *P. salmonis* EM-90 wild and attenuated in Atlantic salmon was used for sRNA candidate selection. First, putative sRNAs *P. salmonis* binding sites in up and down-regulated *S. salar* genes during infection were predicted using RNA22v2.0 software. Two sRNA were selected based on RNAseq analysis expression, synthesized as mimics (mir-222, mir 143-37), and co-transfected with the GFP reporter gene in the salmon head kidney SHK-1 cell line. After 48h, the modified SHK-1 cells were infected with $1x10^6$ CFU/mL *P. salmonis*. Cytotoxicity and cytopathic effects were monitored 24 hours after infection. RT-PCR analysis indicated regulation of transcription of immune-related genes in SHK-1 groups transfected with mimics. Moreover, an increase in the relative expression of the E3 ubiquitin-protein ligase CBL-like gene was observed, suggesting that mir-143-37 could be intervening in the regulation of ubiquitination processes in salmon cells. This study suggests that the pathogenicity process of *P. salmonis* could be regulated through sRNA.

Funding: ANID-Chile through and FONDAP (#15110027) and Ph.D. Scholarship, No. 21191482

POSTERS

P12 Title: Genetic stability of the Primary Cell Bank expressing the recombinant protein active against sea lice in salmon.

Authors: Niuvis Montoya Echavarría[1], Mirlleys Peláez Sánchez[2], Yanelkis Quesada Sarduy[1], Frank Alamos Hernandez[1], Yunier Luis Paneque Diaz[2], Ruhtdali Segura Silva[2], Alain Moreira Rubio[1], Diasmarys Salinas[1], Nemecio González Fernández[2], Irian Mendoza[1], Yamila Carpio[3]

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Abstract:

The recombinant protein active against ectoparasites that affect aquaculture, is produced from the fermentation of the transformed *E.coli* BL21 (DE3) stored at -70 ° C in a pure and homogeneous Primary of Cells Bank (BCP). The objective of the present work is to determine the genetic stability of BCP by obtaining cells resulting from high number of generations and their influence in the growth and expression of the protein. Plasmid stability, analysis of protein expression, restriction pattern and sequencing of the gene of interest was determined to a culture derived from a high number of generations. It is estimated that approximately 54 generations elapse during the production process in a 1000 L fermenter. It was demonstrated that the number of generations did not affect the growth rate (μ) and the doubling time (td) during first Fermentation (F1) μ = 2,37 h-1 and td = 18 minutes, whereas during third Fermentation (F3) μ = 1,69 h -1 and td = 25 minutes. Plasmid stability and protein expression were not affected. The restriction pattern obtained coincided with the expected. It was concluded that the number of generations does not affect the genetic stability of the primary bank.

POSTERS

P13 Title: Iron homeostasis genes modulation in Coho salmon (*Oncorhynchus kisutch*) with jaundice syndrome.

Authors: Cristian Gallardo-Cortes[1,2*], Bárbara P. Benavente[1,2], Valentina Valenzuela-Muñoz[1,2]

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Abstract:

Coho salmon culture in Chile is affected by jaundice syndrome. This syndrome provoked anemia triggers a hematocrit and hemoglobin decrease, altering the hematopoiesis process in fish. The heme group is synthesized during the hematopoiesis using iron available in the organism. These biological alterations suggest the modulation of genes associated with heme biosynthesis and iron transport in sick fish. This study aims to evaluate iron homeostasis-related genes in Coho salmon with significant signs of jaundice syndrome by RT-qPCR analysis. A panel of genes associated with heme biosynthesis and iron homeostasis was evaluated by RT-qPCR analysis. Liver samples were collected from Coho salmon farms. Three different groups of fish were evaluated fish scored has high jaundice syndrome signals (HJS), low jaundice syndrome signals (LJS), and healthy fish (control). The results indicated a significant expression change in evaluated genes among the fish groups. This study exhibited iron homeostasis-related genes modulation in Coho salmon for the first time associated with the jaundice syndrome.

Acknowledgments: This study was funded by CONICYT-Chile through the grants FONDAP (15110027)

POSTERS

P14 Title: Lectin-blotting-2AB: a new sensitive and specific method for screening of lectins from tilapia larvae.

Authors: Bryan A Medina[1], Satomy Pousa[1], Gleysin Cabrera[1], Rebeca Martinez[2]

Affilliations: [†]Department of Proteomics, [£]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba.

Abstract:

Lectins play a key role in the innate immune system of fish to eliminate pathogens. The treatment of tilapia larvae (*Oreochromis* sp) with the Growth Hormone Releasing Peptide-6 (GHRP-6) causes an increased expression of lectin in comparison to untreated larvaes. The identification of differential expression of lectins provides detailed information on the recognition of glycans associated with infectious processes. Also, extraction and identification of lectins from biological sources is challenging due to the presence of cytoplasmic membrane-associated lectins, their low levels of expression and their wide diversity of carbohydrate specificity. In the present work, mechanical methods for protein extraction using an extraction buffer containing guanidinium chloride were employed to obtain the efficient extraction of the total proteins. Protein extracts were quantified by the Microcoomasie method and analyzed by SDS-PAGE. The hemagglutination assay, a traditional method used to lectin detection did not allow checking the presence of lectins in the protein extracts. Consequently, a simple, fast and sensitive method known as Lectin-Blotting-2AB was developed. This method is based on the fluorescence detection of the interaction between lectins and 2-aminobenzamide labelled *N*-glycans. Thus, lectins were detected in treated and untreated protein extracts obtaining a higher expression of lectins in the treated larvae group. Also, lectins with specificity for ribonuclease B, fetuin and asialofetuin *N*-glycans were found in the protein extracts.

POSTERS

P15 Title: Novel biomarkers for smoltification in Atlantic salmon based on non-coding rnas

Authors: Bárbara P. Benavente*, Cristian Gallardo-Escárate and Valentina Valenzuela-Muñoz

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Abstract:

An important phase in the Atlantic salmon life cycle is the smoltification process, which prepares the fish for the parr-smolt transformation. This process is a complex adaptation that consists of several molecular drivers. For instance, in the gills, cortisol and the GH/IGF-1 axis promote the up-regulation of sodium-potassium-ATPase (NaK-ATPase). The NaK-ATPase activity is used in the salmon aquaculture as a smoltification marker, and RT-qPCR for this gene has recently been implemented. However, the NaK-ATPase levels can strongly be altered by hatchery conditions.

This study aimed to explore the putative role of microRNAs during the seawater transfer (SW), and identify novel biomarkers based on non-coding RNAs. For this, Atlantic salmon smolts were exposed to gradual salinity change and gills samples were used for miRNAs mining by Illumina sequencing. A panel of miRNAs with significant expression changes during the SW transfer was validated through qPCR analysis. For the validation, gills samples were obtained during four weeks before the SW transfer. In addition, NaK-ATPase enzymatic activity was evaluated to corroborate the smolt condition.

Herein, we present six candidates biomarkers to evaluate the smolts conditions by qPCR. A significant increase in NaK-ATPase enzymatic activity levels was recorded. Moreover, qPCR analysis showed a significant gradual increase of miR-128, miR-23a-3-5p, and miR-205a-5p during the sampling weeks. On the other hand, miR-205, miR-21a-2-3p, and miR-222 showed a progressive down-regulation during the smoltification. In addition, the target gene analysis evidenced that ATPase-α subunit can be regulated by miR-21a-2-3p. These findings support novel biomarkers for smoltification in salmon aquaculture.

Funding: ANID-Chile through the Postdoctoral grant FONDECYT (#3190320), and FONDAP (#15110027).

POSTERS

P16 Title: Novel molecular markers in the sea louse *Caligus rogercresseyi* associated with hydrogen peroxide treatment

Authors: Constanza Sáez-Vera, Gustavo Núñez-Acuña, Valentina Valenzuela-Muñoz and Cristian Gallardo-Escárate

Affiliation: Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile

Abstract:

The sea louse *Caligus rogercresseyi* is the main ectoparasite species affecting Chilean salmon industry. Hydrogen peroxide (H_2O_2) has been introduced as a delousing treatment to control lice infestations. The suggested mechanism is based on the formation of bubbles in the copepod hemolymph inducing a mechanical paralysis, detaching the parasite from the host. However, the molecular mechanisms underlying the response and potential resistance to H_2O_2 are not yet fully elucidated. This study aimed to expand the molecular knowledge through the single nucleotide polymorphisms (SNPs) discovery involved in the response of *C. rogercresseyi* against H_2O_2.

First, transcription expression of immune-related, antioxidant system, chemosensory receptors, secretome and cuticle formation genes were evaluated in individuals exposed to 3 concentrations of H_2O_2 (360, 744 and 1080 mg L^{-1}). Then, novel SNPs in *catalase, superoxide dismutase, serpin, cuticle 7, cuticle 19* and *trypsin 5* genes were validated in individuals from 4 populations differing in H_2O_2 sensitivity according to bioassays results.

Upregulation of genes related to antioxidant system, secretome and cuticle formation in exposed individuals were observed. Moreover, results from SNPs allele frequencies suggest that alleles of *catalase, superoxide dismutase* and *serpin* genes might explain differences in susceptibility to H_2O_2.

This study contributes to a better understanding of *C. rogercresseyi* responses to H_2O_2, providing new insights into the molecular mechanisms involved in drug resistance. Moreover, the novel SNPs found here could be a potential useful tool for H_2O_2 sensitivity evaluation in lice populations. Further studies will be needed to validate these polymorphisms as a molecular complementary tool for H_2O_2 sensitivity status evaluation. Nevertheless, our investigation will have important implications for H_2O_2 treatment strategies for sea lice.

Funding: ANID-Chile through the grant FONDECYT (#11200813), and FONDAP (#15110027).

POSTERS

P17 Title: PACAP analogs have low toxicity in the RTgut cell line and limited effects on *Aeromonas hydrophila*.

Authors: Laura Rivera[1], Tania Rodríguez-Ramos[1], Lowia Al-Hussinee[1], Yamila Carpio[2], Mario Pablo Estrada[2] and Brian Dixon[1]

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Abstract:

Aquaculture faces the challenge of increasing antibiotic resistance as vaccines are not very effective, so antibiotics are commonly used to control infections. The few available treatments led to the development of new alternative solutions like the use of antimicrobial peptides (AMPs). Pituitary adenylate cyclase activating polypeptide (PACAP) is a prominent member of this wide family, involved in the regulation of antimicrobial activity, growth, immunomodulation, among others functions. Therefore, and thinking in a future oral administration, we decided to test PACAP toxicity in RtgutGC cell line, and to evaluate the effect of PACAP in cultures of *Aeromonas hydrophila*, recognized as a genus with a high pathogenicity in fish and high prevalence in aquaculture facilities. To accomplish these goals, we developed MTT assays for testing RTgut viability in the presence of PACAP at 24, 48 and 72h of incubation, in a range of concentrations from 0.003 to 50.00 uM. Moreover, we determined the Aeromonas hydrophlila growth in presence of PACAP concentrations from 0.05 to 50.00 uM. Our results show that PACAP do not present a considerable toxicity for Rtgut cell line and that do not affect *Aeromonas hydrophila* growth for times evaluated. In general, effects of PACAP sems to be more related with its immunopotentiator role than with the bactericidal activity. Globally the aquaculture industry loses approximately 6 billion USD each year due to diseases outbreaks. These negative effects not only in the economic sector but also in the feeding of the population, indicate PACAP as a possible treatment to save aquaculture losses and prevent antibiotic resistance.

POSTERS

P18 Title: Pituitary adenylate cyclase-activating polypeptide (PACAP) as immunostimulant in juvenile white shrimp *Litopenaeus vannamei*.

Authors: Jesús Luis Betancourt[1], Yamila Carpio[2], Tania Rodríguez-Ramos[3], Mario Pablo Estrada[2], Brian Dixon[3], Laida Ramos[1].

Affiliations: [1]Marine Research Center, Havana University (CIM.UH), Havana, Cuba, [2]Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba, [3]University of Waterloo, Waterloo, Canada.

Abstract:

Disease outbreaks in crustacean aquaculture are emerging due to opportunistic and obligate pathogens and represent severe economic losses to the industry. In this sense, antibiotics are being used as a prophylactic treatment worldwide. However, this overuse and misuse of antibiotics has led to concerning microbial resistance, which has driven the search for novel molecules with immunostimulant and antibacterial activities. PACAP is a multifunctional neuropeptide naturally distributed in a wide range of organisms, and recent studies have shown that it also exhibits immunostimulant and antimicrobial properties in aquatic organisms. Consequently, it is a potential candidate as an alternative to antibiotics in aquaculture. In this work, we evaluate PACAP and three PACAP sequence modified peptides as immunostimulants in juvenile shrimp *Litopenaeus vannamei*. Two bioassays were carried out to evaluate the effect on shrimp immune effectors of one-time administration of PACAP and its variants as well as the effect of different doses with several administrations, respectively. Results showed that PACAP improved hemocyte count and hemagglutination activity of the hemolymph, with effects depending on the modification of the peptide sequence. These findings indicate the potential to use PACAP as an immunostimulant and suggest its use as an option to antibiotics in shrimp cultures.

POSTERS

P19 Title: Production of microalgae for feeding the early stages of white shrimp *Penaeus vannamei* at the YAGUACAM larval production center.

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Abstract:
The production process of microalgae as food in shrimp larval stages, in the UEB YAGUACAM is carried out in the Phytoplankton area that has a strain collection with nine species; renewed every three days in order to maintain their specific characteristics. Of these species, the diatoms *Chaetoceros muelleri* and *Thalassiosira weissflogii* were chosen for production, which are selected independently, from solid medium to 10 ml test tubes; then the process continues with progressive increases in volume from 150 ml to 8000 l with a succession of two cycle days; obtaining daily 16,000 l to 24,000 l with average concentrations of 1000 cel ml-1 and 150 cel ml-1, respectively. Constant monitoring of fundamental variables such as: salinity, temperature, pH, aeration and lighting are carried out. Nutrients that make up the Guillard H medium are applied up to 5 l, while for the rest of the volumes a formulation with agricultural fertilizers is applied, maintaining a Nitrogen: Phosphorus ratio of 17:1; guaranteeing the adequate development of the crop. This food is essential for the nutrition, development and survival of shrimp larvae.

POSTERS

P20 Title: The Atlantic salmon microbiota changes during smoltification

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Abstract:

During the smoltification process, Atlantic salmon (*Salmo salar*) display molecular and physiological changes allowing the parr-smolt transformation. The smoltification process involves metabolic requirements, feeding behavior changes, and osmoregulation process, suggesting strong microbiota modulation. However, a healthy microbiota composition in fish has not been established. Although, it has been suggested that fitness is correlated with increased microbial diversity. This study aimed to explore how the intestinal microbiota of Atlantic salmon can be modulated during the parr-smolt transformation. Pre-smolts Atlantic salmon were transfer to seawater under three different conditions. A group of Atlantic salmon smolts exposed to gradual salinity change (GSC), fish exposed to salinity shock (SS), and a group of fish fed with a functional diet (FD) before the SW transfer. Intestinal samples were collected in freshwater (FW), 10, 32 PSU for the GSC group and at 32 PSU for SS and FD. DNA extraction was performed using the phenol: chloroform isoamyl alcohol protocol. Following this, the entire 16S rRNA gene was sequencing in the Nanopore MinION platform. Then reads analysis was performed with the EPI2ME software package, and BLASTN aligned was performed with the NanoCLUST analysis pipeline. Our results showed an influence of salinity changes in the gut microbiota's richness, diversity, and taxonomic composition, during the smoltification process. Furthermore, the FD fish group's microbiota diversity exhibited high microbiota diversity than the fish exposed to SS. On the other hand, higher richness and diversity were observed in GSC fish than those subjected to the salinity shock group. This study reported the microbiota changes in Atlantic salmon during the seawater transfer for the first time. In addition, it was suggested that a gradual change in salinity and a functional diet could help improve the fitness of fish during the smoltification process in the industry.

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