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CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES

# MikrobioGUNE
# 1st Basque Microbiology Meeting
## Bizkaia Aretoa-UPV/EHU, Bilbao
## December 11th, 2018

MikrobioGUNE
1st Basque Microbiology Meeting

## Program and Abstract Book

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# Program

**9:00 - 9:30** **Registration**

**9:30 - 10:00** **Welcome**

*Arturo Muga (Vicechancellor of Scientific Dev. & Transference - UPV/EHU)*
*Jesús Jiménez Barbero (Scientific Director of CIC bioGUNE)*
Chairs: Aize Pellón (CIC bioGUNE) and Andoni Ramirez-Garcia (UPV/EHU)

**10:00 – 10:45** **Research group presentations (3-min talks) - Biomedicine**

Chairs: Ana Abad (UPV/EHU) and Héctor Rodríguez (CIC bioGUNE)

**BM01** Antimicrobial resistance in livestock

**BM02** Behavior of multidrug-resistant *Acinetobacter baumannii* clinical isolates belonging to IC2 and IC7 in moist conditions

**BM03** *Borrelia burgdorferi* bacterium: A novel contact dependent inducer of peripheral nerve demyelination

**BM04** Fungal infections: the underrated neglected diseases

**BM05** How to manage infectious proteins

**BM06** Innate immune responses to microbes: role of macrophages in homeostasis and inflammation

**BM07** Invasive Fungal Infection Research Group (GEIFI: Grupo Estudio Infección Fúngica Invasora)

**BM08** Plasmid profiles in multirresistent isolations of *Acinetobacter baumannii* obtained in hospitals from Bolivia and the use of phy29 polymerase for its amplification

**BM09** Prognosis biomarkers of listeriosis cases in northern Spain

**BM10** Research lines in the Animal Health Department of NEIKER

**BM11** Research lines in the Microbiology Department of the Hospital Universitarito Donostia-Instituto de Investigación Sanitaria Biodonostia

**10:45– 11:15** **Coffee Break and Poster Session**

**11:15 – 12:15** **Oral communications (12-min talks) - Biomedicine**

Chairs: Ana Abad (UPV/EHU) and Héctor Rodríguez (CIC bioGUNE)

**BM12** Antimicrobial resistance of *Corynebacterium* spp. in the clinical setting

**BM13** Candidiasis and other human infections associated with microbial biofilms

**BM14** Gut microbiome composition in fibromyalgia patients favors higher serum levels of the neurotransmitter glutamate

**BM15** Higher susceptibility of CD4+ T lymphocytes expressing CD300a to HIV-1 infection

**12:15 – 13:00** **Plenary talk: "The Power of Maternal Microbiota on Infant Health"**

*Mª Carmen Collado - Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia*

**13:00 – 15:00** **Lunch time (Iberdrola Tower)**

**15:00 – 15:45** **Research group presentations (3-min talks) - Basic Science**

Chairs: Irati Martínez (UPV/EHU) and Oier Etxebeste (UPV/EHU)

**BS01** BaHiA: Bacterial Hitchhikers Annotation

**BS02** Functional plasmidomics

**BS03** Long-distance signal transduction and the induction of asexual spore formation in filamentous fungi

**BS04** Molecular basis of copper homeostasis in the ascomycota *Aspergillus nidulans*
**BS05** Molecular insight of the early asexual development regulator FluG in *Aspergillus nidulans*
**BS06** RbfA drives ribosome assembly by controlling the maturation state of the 30S decoding center
**BS07** Structural studies on novel translational inhibitors: toward the development of antibiotics with a new mechanism of action
**BS08** Transfer of nucleoprotein complexes into human cells through bacterial Type IV Secretion Systems
**BS09** Structural and Immunological Studies of Schmallenberg and Bovine Viral Diarrhea Viruses
**BS10 (POSTER ONLY)** Flavivirus as probes to study *Brucella* effector proteins

## 15:45 – 16:30 Oral communications (12-min talks) – Basic Science

Chairs: Irati Martínez (UPV/EHU) and Oier Etxebeste (UPV/EHU)
**BS11** Genetics of bacterial biofilm formation process
**BS12** Magnetotactic bacteria and magnetosomes: promising biomedical tools
**BS13** Molecular mechanism for the subversion of the retromer coat by the *Legionella* effector RidL

## 16:30– 17:00 Coffee Break and Poster Session

## 17:00 – 17:45 Research group presentations (3-min talks) - Environment and Food

Chairs: Ilargi Martínez (UPV/EHU) and Maite Orruño (UPV/EHU)
**EF01** Analysis of bacterial stress responses associated with climate change
**EF02** Inhibitors of bacterial conjugation, new strategies for the control of antibiotic resistance in the environment
**EF03** Marine Microbiology through molecular lens: From ecology to applications
**EF04** Metagenomics in beekeeping, viticulture and animal food production
**EF05** Presentation of the Soil Microbial Ecology Group from NEIKER - Basque Institute of Agricultural Research and Development
**EF06** Gut Microbiomics and Membrane Lipidomics as molecular approach for precision nutrition
**EF07** Molecular epidemiology of *Campylobacter* and *Arcobacter*
**EF08** Molecular microbiology in environment, food and clinical settings
**EF09** Selection and improvement of microbial starters for the fermentation industry
**EF10** Strategies to improve heterologous synthesis of docosahexaenoic acid in *Escherichia coli*

## 17:45 – 18:30 Oral communications (12-min talks) - Environment and Food

Chairs: Ilargi Martínez (UPV/EHU) and Maite Orruño (UPV/EHU)
**EF11** Bacteriophage biocontrol of foodborne pathogens and food spoilage bacteria
**EF12** Biological remediation of contaminated soils aimed at soil health improvement
**EF13** Marine microbiology through molecular lens: from ecology to applications

## 18:30 – 18:45 Concluding Remarks and Discussion

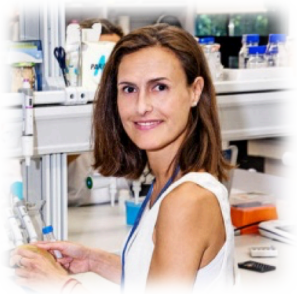
Aize Pellón (CIC bioGUNE) and Andoni Ramirez-Garcia (UPV/EHU)

# Plenary talk

# Mª Carmen Collado

*Institute of Agrochemistry and Food Technology (IATA-CSIC)*

## BioData



**Mª Carmen Collado**, PhD (Polytechnic University of Valencia (UPV), Valencia, Spain, 2005); Research Scientist at Dept. Biotechnology, Institute of Agrochemistry and Food Technology (IATA) of the Spanish National Research Council (CSIC) located in Valencia, Spain. Her research work is multidisciplinary and includes microbiology, food science and nutrition areas. Her interests are focused on probiotics, microbiota and health and nutrition. Her current work includes basic and applied research on molecular analysis and evaluation of health effects of beneficial bacteria and probiotics, the microbial-host interactions, microbiome and its role in human health and diseases and also, the influence of diet (lactation) and other perinatal factors.

**"The Power of Maternal Microbiota on Infant Health"**

The advances in the understanding of the host–microbes interactions suggest that maternal microbiota plays a crucial role in infant health. Microbial colonization is essential for the immune system development and function. Accumulating evidence suggest that the human microbial contact begins in utero and later, it is driven and modulated by perinatal factors including mode of delivery and infant diet. Maternal microbiota forms the first and unique microbial inoculums, and from birth onwards, the microbial diversity increases and converges towards an adult-like microbiota by the end of the first years of life. Alterations in this microbial colonization process, which includes a delayed microbial colonization, alterations in the microbiota profiles and lower microbial diversity in early life, are strong risk factors for the development of some diseases during life. Taken all information available, an adequate nutritional and microbial environment during the perinatal period is key in promoting and supporting human health.

# Topic 1:

## Biomedicine

# BM01

## Antimicrobial resistance in livestock

*Natalia Elguezabal, Beatriz Oporto, Ana S. Tedim, Gorka Aduriz and Ana Hurtado*

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The emergence and spread of bacteria resistant to several (multi-resistant), and sometimes all (pan-resistant) antimicrobials available for treatment has become a global threat and a socio-economic challenge. The selective pressure associated to the overuse of antimicrobials, both in humans and in animals, accelerates the problem of antimicrobial resistance (AMR). In particular, the use of antimicrobials in food-producing animals increases the occurrence of resistant bacteria in animals and on food of animal origin thereby increasing human exposure to these resistant bacteria. With the final aim of reducing antimicrobial use in food-producing animals, the Animal Health Department of NEIKER works in the development of strategies to prevent infections in livestock production through the implementation of integrated management and health programs and the development of immune modulators to boost animal immune response. Phenotypic profiles and genetic determinants of resistance in bacteria from food-producing animals are also studied to describe the epidemiology and mechanisms of antimicrobial resistance.

## BM02

# Behavior of multidrug-resistant *Acinetobacter baumannii* clinical isolates belonging to IC2 and IC7 in moist conditions

*Nadet Manterola, Mónica Cerezales, Itziar Alkorta, Lurdes Sarria, Zulema Bustamante and Lucía Gallego*

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In the last decades, *Acinetobacter baumannii* has become one of the most relevant pathogens and it is responsible for many outbreaks in hospitals worldwide. Controlling these infections is extremely difficult due to its capacity to survive in the hospital settings and its increasing antimicrobial resistance. *A. baumannii* isolated from moist hospital environment such as taps or showers has been described lately, as well as hospital residual waters, water treatment plants or other natural aquatic environments. This data mirrors the dissemination of clinical isolates from the hospital to the environment and upside down. However, there is no data of *A. baumannii* survival in moist conditions. In this work, it was studied the behavior in tap water of two clinical isolates of *A. baumannii* belonging to IC2 and IC7, with counting of colony-forming units up to day 40 and presence of viable cells for a longer time. In addition, changes in the antimicrobial resistance profiles were observed along the study period, what could be due in part to the loss of plasmids that the isolates underwent, the isolates were more susceptible to the majority of the isolates at the end of the experiment. Additionally, CHROMagar *Acinetobacter* (Chromagar, Paris, France) cultures showed motility phenotypes. All these changes could be due to stress adaptations, favoring their survival. This study showed the great ability of *A. baumannii* to persist and survive in moist conditions.

Key words: *Acinetobacter baumannii*; IC2; IC7; survival; water; antibiotic resistance.

## BM03

# *Borrelia burgdorferi* bacterium: A Novel Contact Dependent Inducer of Peripheral Nerve Demyelination

*Marta Palomo Irigoyen[1], Encarnación Pérez Andrés[1], Miguel Tamayo Caro[1], Adrián Barreira Manrique[1] and Ashwin Woodhoo[1,2]*

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Schwann cells form myelin sheaths around large diameter axons in the peripheral nervous system, and this is essential for rapid saltatory conduction of nerve impulses. Myelin breakdown, demyelination, is a universal outcome of a remarkably wide range of conditions that involve disturbance to Schwann cells or the nerve environment, whether due to genetic or acquired disease, toxicity or microbial infections. Strikingly, demyelination is often seen in Lyme neuroborreliosis (LNB), which is considered one of the most dangerous of all manifestations of Lyme disease, the most common arthropod-borne infectious disease in temperate regions of the northern hemisphere. It is caused by infection with the spirochete *Borrelia burgdorferi* (Bb). The early local reaction to the deposition of the bacteria in the skin is followed by the hematogenous dissemination of the spirochete, which results in the colonization of different tissues and organs such as the skin, nerves and brain. In this study, using in vitro analyses, we show that Bb is a potent contact-dependent inducer of peripheral nerve demyelination, by activating several receptor and intracellular signalling pathways to strongly repress myelin gene and protein expression, throwing light on one of the most intriguing pathological features of Lyme neuroborreliosis.

# BM04

## Fungal infections: the underrated neglected diseases

*A. Ramirez-Garcia, A. Antoran, I. Buldain, X. Guruceaga, L. Martin-Souto, L. Aparicio, U. Perez-Cuesta, M. Areitio, S. Cendon, A. Abad-Díaz-de-Cerio, A. Rementeria and F.L. Hernando*

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The incidence of fungal infections is increasing globally in the last years. In fact, infections caused by microscopic fungi affect millions of individuals, mainly immunocompromised, every year with unacceptable mortality rates, which usually exceed 50%. The main factors that causes these fatal results are the delay in diagnosis due to the lack of rapid, specific, and sensitive detection methods and the resistances of many of these fungi to the commonly used antifungal drugs.

Therefore, the Fungal and Bacterial Biomics Research Group from the University of the Basque Country (UPV/EHU) focuses its efforts on shedding light on the pathobiology of the most important fungal pathogens, mainly *Candida*, *Aspergillus*, *Scedosporium*/*Lomentospora*, with the aim of increasing the understanding of the virulence mechanisms. Currently, the group is mainly researching into the following three lines:

On the one hand, we study the most prevalent airborne pathogenic filamentous fungus, *Aspergillus fumigatus*. Our purpose is to delve into the knowledge of the infection by *A. fumigatus* and contribute to the general knowledge of this fungus. For that, whole genome transcriptomic studies using AWAFUGE v.1 microarray, a whole genome custom microarray designed by us, have deepened in the genomic expression dataset allowing us to know more about its virulence mechanisms and select new therapeutic and diagnostic targets. In addition, other techniques such as infections into different animal (mouse and *Galleria mellonella*) and cell line models, immunological and histological techniques, expression analysis using RT-qPCR, mutant strains generation, sequencing and bioinformatics are normally used by our group.

On the other hand, we search for the identification of new diagnostic and therapeutic targets of the group *Scedosporium*/*Lomentospora*. Of particular concern is their high resistance to antifungal treatments and, therefore, they cause very high mortality rates in immunosupressed patients. Moreover, they are the second filamentous fungi in cystic fibrosis patients, only behind *Aspergillus*. To do that, we use several technologies such multidimensional electrophoresis and mass spectrometry that allow us the identification and characterization of the most interesting targets. Currently, we are designing a serologic method to detect *Scedosporium*/*Lomentospora* in cystic fibrosis patients and monitorize them.

Finally, the group has been studying for several years the role of *Candida albicans* on tumor adhesion. In this sense, we have demonstrated that the inflammatory response produced by *C. albicans* in the hepatic endothelium favors the adhesion of tumor cells to the endothelial cells, leading to liver metastasis in vitro and in vivo. Furthermore, we identified several molecules as putative candidates to be enhancers of the response and receptors involved in the process. We have also produced monoclonal antibodies to inhibit the effect of the identified molecules and currently we are also studying the role of the bacteria that most frequently cause

nosocomial infection, *Escherichia coli* and *Staphylococcus aureus* have in carcinogenesis and metastasis.

Therefore, our group places special emphasis on the characterization of the cellular, molecular and genetic bases involved in the genesis and development of different fungal diseases from a multidisciplinary approach, which includes the development of a variety of technologies, such as "omics", and their application to the study of pathology.

# BM05

## How to manage infectious proteins

*Hasier Eraña[1,2], Jorge M. Charco[1], Sandra García-Martínez[1], Rafael López-Moreno[1], Ezequiel González-Miranda[1], Miguel A. Pérez-Castro[1], Carlos M. Díaz-Domínguez[1] and Joaquín Castilla[1,3]*

[1]CIC bioGUNE, Parque tecnológico de Bizkaia, Derio, Bizkaia, Spain. [2]ATLAS molecular pharma, Parque tecnológico de Bizkaia, Derio, Bizkaia, Spain. [3]IKERBASQUE, Basque Foundation for Science, Bilbao, Bizkaia, Spain.

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The term prion was coined by Stanley Prusiner to define a new biological agent that based its infectious behavior on its natural capacity for self-replication. Although this characteristic is shared by many other infectious agents, the peculiarity of this new pathogen was that it was composed of a single protein. This biological "heresy" referred to as the "protein only" hypothesis attributed to a single protein the ability to show a great diversity of different structures with perfectly differentiable pathogenic properties. Prions are unique infectious proteins capable of infecting animals and humans resulting in a fatal neurodegenerative disease.

Our laboratory works with this peculiar infectious agent trying to understand the three most important characteristics that make prion diseases unique: infectivity, strain and transmission barrier phenomena. These aspects are being studied using sophisticated in vitro systems able to efficiently propagate infectious prions.

Their unusual property of perpetuating their structure by self-replicating mechanisms must be governed by a specific structural code. Decryption of this code has become the Holy Grail in prion biology, as it would definitely ensure the understanding of all aspects of misfolded protein propagation. At this moment, the only technique able to unravel the structure of aggregated proteins with an atomic resolution is solid-state NMR (ssNMR). However, it has two key limitations: it needs isotopically labeled protein and requires quantities currently unattainable by current in vitro prion production methods. Thus, we have developed a new methodology able to produce quasi-unlimited amounts of isotopically labeled infectious material that will allow us to tackle resolution of the structure of prions, and this, in turn, to fully understand its infectious nature.

# BM06

## Innate immune responses to microbes: role of macrophages in homeostasis and inflammation

*Leticia Abecia, Estíbaliz Atondo, Diego Barriales, Ana Carreras-González, Itziar Martín-Ruiz, Marta Montesinos-Robledo, Ainhoa Palacios, Miguel Ángel Pascual-Itoiz, Aize Pellón, Ainize Peña, Rafael Prados-Rosales, Héctor Rodríguez, Leticia Sampedro and Juan Anguita*

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Macrophages are key components of the immune response to microorganisms and have been traditionally established as first-line responders to infectious agents with a dual role eliminating pathogens by phagocytosis while mediating defensive and inflammatory pathways. Moreover, recent research points to these immune cells as key players in the modulation of the populations of commensal and health-promoting microbes.

Our lab seeks to understand the response of macrophages to infectious agents, from Lyme borreliosis to microbiota-related disorders such as inflammatory bowel disease (IBD). Among these projects involving pathogens, we are looking for new phagocytic receptors involved in the response to the spirochete *Borrelia burgdorferi*, in terms of bacterial elimination and inflammatory outcome. We are also interested in understanding the metabolic control of macrophage responses through the specific mitochondrial complex I natural inhibitor MCJ in different models of disease in which these cells participate, such as Lyme carditis or IBD. Regarding the role of macrophages in commensal homeostasis, we are also exploring the capacity of these immune cells to sustain beneficial bacteria populations using the probiotic bacteria *Lactobacillus plantarum* as a model. This new macrophage role might have important implications in beneficial bacterial dissemination to extraintestinal sites and homeostatic control of gut ecology.

Recently, our lab has developed a new research line exploring the regulatory capacities of microorganisms in the development of innate immune memory by macrophages, both in the context of pathogenesis (*B. burgdorferi*) and commensalism (*L. plantarum*).

Finally, Anguita´s lab is also performing applied research looking for tick vaccine candidates and also exploring as side-projects hot topics such as microbial metabolite involvement in disease or microbiota implications in Lyme disease. In addition, the research group has created a new scientific platform at CIC bioGUNE, the Monoclonal Antibody Generation Service, to provide customized and reliable antibodies as tools for research.

# BM07

## Invasive Fungal Infection Research Group (GEIFI: Grupo Estudio Infección Fúngica Invasora)

*Inés Arrieta-Aguirre[1] and María Dolores Moragues [1,2]*

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Fungal infections cause a variety of debilitating conditions in humans, animals, and plants. While superficial fungal infections are usually readily treated, invasive fungal diseases (IFDs) that afflict the immunocompromised and individuals with a range of comorbidities have been estimated to kill about 1.35 million people annually (Brown GD eta al, 2012). Among IFDs *Candida* spp. infections represent the fourth cause of nosocomial infections after gram-positive cocci, enterobacteria and non-fermentative GNB, constituting 6-9% of microbial isolates in blood cultures (Peman J & Salavert M, 2013). IFDs caused by *Candida* and *Aspergillus* are the most frequent in immunocompromised patients. Nevertheless there exist a growing number of fungal species that taking advantage of these special conditions cause less common but very serious infections with a high mortality rate. The study of infections caused by a wide and taxonomically diverse array of opportunistic fungi has made the field of medical mycology a great challenge (Pfaller MA & Diekema DJ, 2004).

Regarding *Candida*, mortality rates associated with invasive candidiasis remain high (30% to 40%) (Lortholary O et al., 2014), so it is important to initiate antifungal therapy as soon as possible to select the most appropriate antifungal agent in each case. These infections are mostly treated with azoles, mainly fluconazole (Morio F et al., 2013), fungistatic drugs that inhibit growth, but do not kill the fungus, thereby providing the opportunity for resistance development (Morschhäuser J et al., 2016).

In order to broaden knowledge of these pathogenic fungi and to provide new tools for diagnosis, prevention, allowing an earlier and specific treatment, and with lower healthcare cost for these infections, our Invasive Fungal Infection Research Group addresses four main lines of research:

1. Isolation and characterization of fungal species involved in human pathology. Molecular diagnosis of antifungal resistance.

2. Identification of new markers for serological diagnosis of invasive candidiasis.

3. Identification of new components useful for immunoprophylaxis of fungal infections.

4. Characterization of C7 and Cg26 monoclonal antibodies, that react with *Candida albicans* and other fungal species.

Brown GD et al. 2012. Science. 336-647.
Peman J & Salavert M. Enferm. Infecc. Microbiol. Clin. 2013. 31:328-341.
Pfaller MA & Diekema DJ. 2004. J. Clin. Microbiol. 42:4419-4431.
Lortholary O et al. 2014 Intensive Care Med. 40:1303-1312.
Morio F et al. 2013. Int. J. Antimicrob. Agents. 42:410-415.
Morschhäuser J. 2016. J. Microbiol. 54:192-201.

## BM08

# Plasmid profiles in multirresistant isolations of *Acinetobacter baumannii* obtained in hospitals from Bolivia and the use of phy29 polymerase for its amplification

***Silvia Pérez, Mónica Cerezales, Carla Ferrero, Alazne Calderón, Ohiane Pérez, Elena Fernandez-Colón, Zulema Bustamante, Itziar Alkorta and Lucía Gallego***

*Acinetobacter baumannii* Research Group; Department of Immunology, Microbiology and Parasitology; Faculty of medicine and nursing; University of Basque Country

*Acinetobacter baumannii* is an opportunistic pathogen responsible for severe nosocomial infections in hospitalized patients with compromised immune systems. In recent years the resistance of strains of *A. baumannii* to antibiotics has increased, this has limited the treatment alternatives, compromising the health of patients and producing a major problem worldwide. Plasmids have a fundamental role in the acquisition and dissemination of virulence genes, however, the information about these is scarce in Latin American countries such as Bolivia. In this work a commercial kit was used to characterize the different plasmid profiles of strains of *A. baumannii* from the Materno-Infantil Hospital, Cochabamba (Bolivia), obtained during 2014/2015 and this profiles were compared with previous studies to observe the evolution of the clinic situation. In addition, the use of Polymerase Φ29 is proposed to amplify the plasmids for future sequencing.

Key words: *Acinetobacter baumannii*, opportunistic pathogen, multiresistance (MDR), plasmid, plasmid profile, carbapenems, polymerase Φ29.

# BM09

## Research lines in the Animal Health Department of NEIKER

*Joseba M. Garrido, Gorka Aduriz, Iker S. Agirregomoskorta, Marta Alonso, Marta Barral, Natalia Elguezabal, Ana García and Ana Hurtado*

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NEIKER is an entity owned by the Department of Economic Development and Infrastructure from the Basque Government that works close to the agrifood industry. The agrifood industry faces continuous challenges that at this moment are conditioned by an increase in population demanding food supply in quantity and quality. Our department's main role is to offer solutions to the farmers that go from farm management advice and animal disease diagnostic services to proper research and innovation projects. These projects are aimed at improving the rentability of the farms, assessing the food security working at farm level and to reducing the impact of animal diseases or vectors that could affect Public Health.

Our three research areas and the main diseases or infections in which we focus our activity are:

1. Diagnosis, control and epidemiological surveillance of animal diseases.
   a. Mycobacteriosis: Paratuberculosis and Tuberculosis (Tb)
   b. Reproductive diseases: Neosporosis, Campylobacteriosis, Toxoplasmosis, Bovine Viral Diarrhea and Border Disease
   c. Vector-borne diseases: Piroplasmosis and Anaplasmosis
   d. Q Fever
   e. Viral diseases: Influenza, Maedi-visna, Bluetongue
2. Zoonosis and food safety
   a. Antimicrobial resistance control strategies and alternatives to antimicrobials in farm animal production
   b. Epidemiology of food-borne infections (*Salmonella* spp., *Listeria* spp., *Campylobacter* spp. and *E. coli*) in livestock
   c. Diagnosis, control and epidemiology of zoonosis like Q fever, Echinococcosis, Transmissible Spongiform Encephalopathies, Bovine Tb
3. Environmental biosecurity, wildlife and vectors as a source of human and livestock infections

Most of our research and diagnostic service is performed in our facilities that include both BSL2 and BSL3 laboratories and animal facilities. However, an important part of our research is performed at the farms and abattoirs (livestock) and in the field (wildlife species). Laboratories are fully equipped and divided in different areas where molecular biology, tissue culture, immunology, microbiology, parasitology, histopathology and data analysis techniques are covered permitting integrated studies that are aimed at providing solutions to animal diseases. These include the development of new diagnostic reagents, development of immunomodulators (vaccines, probiotics, antibodies, etc.), molecular characterization of microorganisms, disease transmission modelling at the human-livestock interface and the generation of knowledge to elucidate pathogenic mechanisms that will help better understanding of the diseases and their etiological agents, aiding in the discovery and development of innovative solutions for control and eradication of animal diseases. The animal

facilities are mainly used for animal model studies aimed at studying pathogenesis and evaluation of new therapeutic products for veterinary medicine.

# BM10

## Research lines in the Microbiology Department of the Hospital Universitarito Donostia-Instituto de Investigación Sanitaria Biodonostia

*Ainara Arana Salaverría, Jose María García Arenzana Anguera, María Gomáriz Díaz, Milagrosa Montes Ros, Luis Darío Piñeiro Vázquez, Marta Alonso Asencor, María Ercibengoa Arana, María Fernandez-Reyes Silvestre, Pedro Idígoras Viedma, Jose María Marimón Ortiz De Zárate and Diego Vicente Anza*

Servicio de Microbiologia, Hospital Universitario Donostia-Instituto de Investigación Sanitaria Biodonostia

The research on infectious diseases performed by our group is focused on the epidemiology of different bacterial and viral infections (incidence, evolution through time, clonal distribution, etc.) as well as on the prevalence, changes and mechanisms of antimicrobial and antiviral resistance detected. The rapid diagnosis of pathogenic microorganisms and the detection of mechanisms of resistance will result in a clinical and economic optimization of antibiotic treatments, since antibiotics will be used more rationally and the therapeutic failures of resistant isolates will be avoided.

The prevention of infections by vaccination is probably one of the grater achievements of medicine. Our group focuses his studies on the consequences of introduction of Rotavirus, pneumococcal or meningococcal new vaccines in our region.

More specifically, the objectives of the three lines of research are:

1. Respiratory infection:
   a. Epidemiology of *Streptococcus pneumoniae* Infections.
   b. *Streptococcus pyogenes* infections: clinical impact and molecular epidemiology.
   c. Respiratory infection caused by viruses and less common bacteria (*Nocardia* and *Coxiella*).
   d. Microbiome alterations in severe community acquired pneumonia.
2. Antimicrobial resistance:
   a. Bacterial resistance in respiratory pathogens.
   b. Multi resistance in hospital acquired infections.
   c. Bacterial resistance and epidemiology of zoonotic pathogens: *Campylobacter*, *E. coli*, *Salmonella*, ...
   d. *Helicobacter pylori* infection and antimicrobial resistance.
3. Vaccination preventable diseases
   a. Rotavirus. Evaluation of the introduction of new anti-rotavirus vaccines. Impact on the child population of the Basque Country.
   b. Invasive meningococcal disease. Impact of vaccination with conjugate vaccine. Usefulness of the new *N. meningitidis* group B vaccines.
   c. Prevention of cervical carcinoma and *Chlamydia* infections: selection of the most appropriate screening methods, prevalence of persistent papillomavirus infections and causal genotypes.
   d. Surveillance of other emerging and re-emerging diseases and evaluation of recently established vaccination programs.

# BM11

## Vaccines for listeriosis

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Introduction: Despite its increase in Europe during the last years, there is no vaccine approved against listeriosis. The goal of our laboratory has been the development of a prophylactic therapy that may be not only effective against the disease, but also easy and cheap to implement in healthcare systems.

Materials and Methods: While several *in silico* and *in vitro* experiments were made to search for peptides to be used in vaccination and to study its capacity to develop an immune response and its safety, the most useful tool in our research was the use of animal models. They allowed us to study the effectivity of our vaccines in models of different susceptibility to develop the disease, as well as listeriosis infection during pregnancy.

Results: The use of a cellular vector like dendritic cells allowed us to see that two peptides of *L. monocytogenes* LLO and GAPDH virulence factors have a great effectivity in the prevention of listeriosis, providing an effective Th1 immune response. As cellular therapy may be too expensive and difficult to apply on clinical practice, we use another vector: gold glyconanoparticles. These nanovaccines, used with adjuvants, proof to be as effective as dendritic vaccines, providing great protection levels in all the murine models we used.

Conclusions: During these years our laboratory has developed two different kinds of vaccines against listeriosis. While dendritic vaccines could be used in some specific cases like oncological patients, the use of nanovaccines may be a more convenient preventive therapy for the vaccination of global population.

# BM12

## Antimicrobial resistance of *Corynebacterium* spp. in the clinical setting

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*Corynebacterium* species are widely distributed in the environment and in the microbiota of humans and animals. Medically relevant *Corynebacterium* species include *Corynebacterium diphtheriae*, the primary cause of diphtheria, and the non-diphtherial corynebacteria, which are part of the normal flora of the skin and mucous membranes. Nondiphtherial corynebacteria have been frequently dismissed as a contaminant when isolated from clinical materials. However, the role of these bacteria in clinical disease is now more clearly established. They are actually recognized as causing opportunistic disease, particularly in specific circumstances, such as repeated exposure to broad-spectrum antibiotics, after the use of invasive medical procedures or in patients who have suffered long-term hospitalization.

Hospitals are “antibiotic-rich” environments, creating a selective pressure on the survival of resistant bacteria by which resistance genes can be transferred to susceptible bacteria, spreading multi-resistance. In recent studies including isolates belonging to two clinically relevant *Corynebacterium* species[1,2], we have observed the high incidence of multi-resistance, in particular to beta-lactamics, quinolones and macrolides. Resistance mechanisms were elucidated through genomic analysis and phenotypic characterisation of a group of selected strains.

1. Navas J, Fernández-Martínez M, Salas C, Cano ME, Martínez-Martínez ME. 2016. Susceptibility to aminoglycosides and distribution of aph and aac(3)-XI genes among *Corynebacterium striatum* clinical isolates. PLOS One 2016; 11(12): e0167856.

2. Alibi S, Ferjani A, Boukadida J, Cano ME, Fernández_Martínez M, Martínez-Martínez L, Navas J. 2017. Occurrence of *Corynebacterium striatum* as an emerging antibiotic-resistant nosocomial pathogen in a Tunisian hospital. Sci. Rep. 7, 9704. doi: 10.1038/s41598-017.

# BM13

## Candidiasis and other human infections associated with microbial biofilms

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The Consolidated Research Group of the Basque University System "Candidiasis and other human infections associated with microbial biofilms" (GIC15/78 IT-990-16) is part of the Multidisciplinary Training and Research Unit 11/25 "Microbes and Health" of the University of the Basque Country/Euskal Herriko Unibertsitatea (UPV/EHU). Lecturers, research staff and clinical professionals compose the Group. This interdisciplinary and transversal character is one of its strengths. The main objective of our Group is to broaden knowledge on the pathogenesis of candidiasis and other human infectious diseases associated with the production of microbial biofilms and their relationships with different pathological processes. The following are among the specific objectives:

1) To update the epidemiology of candidiasis and other infections associated with the development of biofilms.
2) To deepen the knowledge of microbial biology and the pathogenesis of infections caused by pathogens that develop biofilms.
3) Improve and develop technologies that allow a quick and correct diagnosis.
4) To evaluate the pharmacokinetic and pharmacodynamic (PK/PD) properties of antimicrobials and the molecular bases of microbial resistance to these drugs.
5) To search for possible preventive and therapeutic alternatives that improves the prognosis of these infections.

The Group is structured in three interdependent lines of research:

1) "Epidemiology, pathogenesis and diagnosis of candidiasis and other infections associated with biofilms" (director: Guillermo Quindós). The line focuses on the study of the pathogenesis of candidiasis and other diseases associated with biofilms, with the development of the methodology for studying microbial biofilms in different biomaterials, the development and assessment of the methodology for diagnosing these infections based on differential isolation, biochemical, enzymatic or immunological properties, DNA probes or molecular methods for the identification and epidemiological typing of clinical isolates of pathogenic microorganisms.

2) "*In vivo* models for the study of pathogenicity and susceptibility to antimicrobial drugs of *Candida* and other biofilm producing pathogens" (director: Elena Eraso). This line focuses on the use of experimental models in invertebrate animals for the study of the virulence of *Candida*, the evaluation of the efficacy of drugs in the treatment of mycosis and the search for

therapeutic alternatives. *Galleria mellonella* and *Caenorhabditis elegans* present advantages over mammalian models due to their easy maintenance, the use of a significant population with a low cost, small size or short life.

3) "Pharmacokinetic/pharmacodynamic models *in vitro* to determine and predict the efficacy of antimicrobial drugs against emerging species of *Candida* and other biofilm producing pathogens" (director: Nerea Jauregizar). This line focuses on obtaining time-lethality data in vitro and subsequent FC/FD analysis of antifungal drugs, especially those of more recent incorporation such as echinocandins. In addition, the line develops PK/PD models to describe and prevent resistance to antimicrobial drugs.

Our Group receives funding from different national and international entities and institutions, it has published more than 222 scientific manuscripts, and it actively contributes to the dissemination of Science through different media and social networks. In addition, a priority task of the Group is the training and hiring of new researchers and professors promoting the completion of master's degrees, doctoral theses, continuing education courses and research stays.

## BM14

# Gut microbiome composition in Fibromyalgia patients favors higher serum levels of the neurotransmitter glutamate

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Fibromyalgia is a complex and relatively unknown disease which main symptomatology includes chronic pain. Gut-brain axis connects the gut microbiome and brain through the enteric nervous system (ENS) and its disruption has been associated with both psychiatric symptoms and gastrointestinal disorders. Here, we combined metagenomics and metabolomics to provide an insight into fibromyalgia pathogenesis and potential diagnostic biomarkers. 105 fibromyalgia patients were recruited together with 54 healthy controls. All of them provided faeces samples and signed informed consent. Amplicons of V3 plus V4 16S-rDNA region from faeces were sequenced with MiSeq (Illumina). Samples were processed with QIIME2. Taxonomy identification was done with GreenGenes database. Diversity measurements and differential composition were estimated and identification of altered metabolic pathways was inferred using Picrust. Serum samples from same individuals were analysed by high-resolution liquid chromatography-coupled Time-of-Flight mass spectrometry. Microbiome multivariate analysis (PLS-DA) allowed the complete discrimination of fibromyalgia patients from controls (p=0.0019). Alpha diversity indexes showed a reduction in biodiversity in fibromyalgia patients respect to controls (Faith's PD p=0.04). Significantly, *Bifidobacterium* genus was found to be decreased in fibromyalgia patients. Other bacteria genera were reduced in fibromyalgia patients, consistent with gut dysbiosis events. Serum metabolomics showed alterations in metabolites related to the conversion of glutamate to gamma aminobutyric acid (GABA), thus indicating that this metabolic pathway could be altered in fibromyalgia patients. Specifically, we found that glutamate and its precursors were elevated in patient's sera, while GABA was decreased. Notably, one of the *Bifidobacterium* functions is the conversion of glutamate to GABA. The role of glutamate and its receptors on the development and maintenance of neuropathic pain has been previously described, as the role of GABA as pain inhibitor. Our study shows how the integration of microbiome and serum metabolomics data can provide insights into fibromyalgia's disease pathogenesis.

# BM15

## Higher susceptibility of CD4+ T lymphocytes expressing CD300a to HIV-1 infection

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Introduction: CD300a is known to have an important role in several viral infections; regarding the Dengue virus, the binding of the receptor with its ligands promotes the infection of host cells. We recently described that CD4+ T cells from HIV-1 infected patients displayed higher levels of CD300a than the ones from healthy donors. The main objective of the following study was to study the susceptibility of CD300a expressing CD4+ T cells to HIV-1 infection, in comparison with CD300a- population.

Methods: Memory CD45RA- CD4+ T cells were purified from peripheral blood mononuclear cells from healthy donors. Cells were infected with HIV (BaL, MOI 240:1) and cultured with interleukin-2 (IL2) during 7 days. Furthermore, memory CD4+ T lymphocytes from cART naïve HIV-1 infected patients (n=6) were also purified and cultured with IL2 during 13 days. Flow cytometry was utilized to measure the percentage of infection (anti-p24) and the expression of CCR5, CD38, HLA-DR and PD1 on CD300a+ and CD300a- CD4+ T cells.

Results: Before the infection, CD300a+ CD4+ T cells exhibited higher percentage of CCR5+ cells, but lower percentage of CD38+, HLA-DR+ and PD1+ cells, when we compared with CD300a- cells. Nevertheless, after HIV infection, CD300a+ CD4+ T cells displayed a higher expression of CCR5, CD38, HLA-DR and PD1, than CD300a- cells. Importantly, we observed a higher number of infected cells (p24+) within CD4+ T lymphocytes expressing CD300a, in comparison with CD300a- cells. Finally, we saw that endogenously HIV infected CD4+ T cells belonged to HIV-1 infected patients expressed higher levels of CD300a than non-infected cells.

Conclusions: CD4+ T lymphocytes expressing CD300a exhibit a higher expression of activation markers and CCR5 after the infection with HIV. Moreover, CD4+ T cells expressing CD300a are more susceptible to HIV infection than cells negative for the receptor.

# Topic 2:

## Basic Science

# BS01

## BaHiA: Bacterial Hitchhikers Annotation

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In the last decade, different evidences suggest the possibility of bacterial translocation through the host body. This would explain the presence of bacteria in body niches previously considered sterile, such us the breastmilk. Different research results seem to indicate that gut bacteria, somehow, are able to translocate from the mother's gut to the milk, being the most accepted pathway for this microbial trip the one involving immune cells as vehicles for bacteria. Although, the microbial mechanisms for translocation have not been deciphered yet, we hypothesize that to travel within immune cells, microbial "travellers" should be able to survive within these vessels in a similar way to the one employed by intracellular pathogens.

To find the genetic patterns that characterize translocation we selected a set of genes employed by well-characterized intracellular pathogens to internalize inside immune cells. Then, we performed comparative genomic analyses to find the orthologues of these genes among the genome and proteome sequences of a series of bacterial strains. Finally, the genetic patterns correlated with translocation were detected using the latest unsupervised machine learning (UML) algorithms.

Here we present BaHiA (Bacterial Hitchhikers Annotation) that provides a prediction tool on the bacterial translocation probability. BaHiA uses BLAST (BLASTp or BLASTn) to find regions of similarity between input sequences and the server's BLAST protein database. These results are then processed through supervised machine learning (SML) algorithms to classify the input sequence as a potential translocating organism or not.

This is the first tool designed to perform microbial translocation prediction analysis using machine learning algorithms. This tool provides a user-friendly interface that can be used without any bioinformatics knowledge through a single submission step. In the end, BaHiA provides a set of downloadable results like images and tables in plain text format that can be easily understood and further processed by the user.

# BS02

## Functional plasmidomics

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The Intergenomics group is interested in several aspects of the horizontal gene transfer mediated by plasmids. Some of our current research lines deal with the natural and artificial strategies to control plasmid spread through conjugation, the transcriptional regulatory networks of conjugative plasmids, plasmid ecology, and the engineering of synthetic biology circuits for distributed bacterial computations using plasmids as communication devices. We have developed some bioinformatics tools to: i) reconstruct plasmid sequences from Illumina data (PLACNET), ii) analyze plasmid mobility (MOBscan), and iii) perform comparative plasmid genomics (AcCNET and pANI).

## BS03

# Long-distance signal transduction and the induction of asexual spore formation in filamentous fungi

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Filamentous fungi are widely used in industry as a source of compounds such as antibiotics, immunosuppressants or organic acids. They have also established both symbiotic and antagonistic associations with humans, animals and plants, having a significant impact on major crop yields and killing thousands of people annually.

Fungal infections in general, and filamentous fungi in particular, are among the most-rapidly spreading pests. Dissemination to new niches is enabled mainly through asexual spores, which can be produced (in their thousands) following different developmental programs. The MBF group (Molecular Biology of Fungi) of the Chemistry Faculty of the UPV/EHU takes advantage of cellular and molecular biology tools so as to study the mechanisms that control asexual spore production in filamentous fungi. With this aim, the model ascomycete *Aspergillus nidulans* is used, the main reference organism in this field. During the last years, we have characterized a signal transduction pathway that plays a key role in the reprogramming of the cell-type involved in substrate colonization, hyphae, in order to generate complex multicellular structures known as conidiophores. Each conidiophore bears thousands of asexual spores called conidia. On new substrates and under the right environmental conditions, conidia germinate and initiate new colonization cycles.

Hyphae have developed a polar (unidirectional) mode of growth, which means that all plasma membrane and cell-wall materials are transported to the growth region or tip. This growth mechanism also implies that the distance between the tip and nuclei is significantly increased, requiring long-distance signal transduction mechanisms to convey to nuclei the information received at the tip and activate the corresponding response pathways. The transcription factor (TF) FlbB is first transported to the tip of hyphae and then basipetally to nuclei. There, in coordination with a second TF, it induces the expression of the first gene of the genetic pathway that controls the generation of conidiophores. Our group has characterized the role of auxiliary proteins, molecular motors, cytoskeletal elements and additional developmental regulators in the intracellular itinerary of FlbB, which also serves as a model for the study of TF-mediated polarity site-to-nucleus communication in other types of polar cells.

## BS04

# Molecular basis of copper homeostasis in the ascomycota *Aspergillus nidulans*

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Cu is an essential cofactor for many biologically relevant enzymes. Thus, high-affinity mechanisms for its acquisition are required. However, excessive copper accumulation results in multiple toxicity effects. The essential, and yet, toxic nature of copper requires precisely controlled homeostatic mechanisms. These include copper uptake, intracellular traffic, storage and detoxification.

Copper homeostasis has been extensively studied in *Saccharomyces cerevisiae*, including the uptake, detoxification and the transcriptional regulation of both processes. Very few studies have been made in filamentous fungi. Since March 2017, however, some articles have been published on copper homeostasis in filamentous fungi, which underscore the importance of copper homeostasis in the development of fungal pathogenicity.

*Aspergillus nidulans* is a model organism which provides the opportunity to learn how the copper homeostasis process functions in filamentous fungi. Mutagenesis studies have revealed several candidates taking part in the different processes involved in copper balance. Two copper transporting (Ctr) proteins involved in copper uptake have been described (AN3209 and AN3813). Various studies revealed that copper transporters are expressed depending on copper availability, and both proteins work in a complementary manner. When it comes to detoxification it was discovered that a P-type ATPase, AN3117, was responsible of copper detoxification in *A. nidulans*, differing from the metallothionein copper detoxification mechanisms described in *S. cerevisiae*. Deletion of AN3117 rendered *A. nidulans* susceptible to copper. Expression studies revealed that AN3117 was only expressed with high environmental copper concentration. A metallothionein coding gene, AN7011, was also discovered, made no contribution to copper detoxification. Two genes coding two transcription factors were identified, AN1924 and AN0658. Mutagenesis and functional characterization studies showed that AN1924 regulates the expression of the copper detoxification system. Deletion of AN1924 further aggravated the copper susceptibility phenotype of the AN3117 deletant strain. On the contrary, AN0658 regulates the expression of the copper uptake system. AN0658 deletion generated a more extreme copper starvation phenotype than the AN3209-AN3813 double deletant strain in copper depleted conditions.

Copper has long been used as an antimicrobial agent because of its broad range of action and the relatively cheap prize compared to other antifungal agents. However, an excessive use has derived in microbial resistance. For this reason, the understanding of the molecular mechanisms of action could provide a valuable insight into new strategies to overcome increasing copper loads for the control of fungal disease in animals and plants.

## BS05

# Molecular insight of the early asexual development regulator FluG in *Aspergillus nidulans*

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*Aspergillus nidulans* is a model filamentous ascomycete which presents a complex developmental pattern combining vegetative growth, asexual and sexual development. This pattern is modulated in response to environmental factors, such as the composition of the medium, light and exposure to the atmosphere.

Genetic analyses employed to examine the transition from vegetative hyphae to asexual development have identified a set of genes that are required to initiate the differentiation process. One of the earliest acting genes is *fluG*, whose deletion results in colonies which fail to produce asexual structures and accumulate aerial vegetative hyphae resulting in a cotton like morphology, commonly known as fluffy.

In this study we applied advanced molecular techniques to unveil the potential enzymatic activity of the FluG protein. Firstly, a bioinformatic examination revealed that FluG is formed by two putative enzymes; the N-terminal region shares sequence and structural similarity with a prokaryotic amidohydrolase (LSEI_0440) from *Lactobacillus paracasei*, and the C-terminal region shares sequence and structural similarity to a g-glutamyl aromatic monoamine ligase (PA5508) from *Pseudomonas aeruginosa*. Expression of the N- and C-terminal regions individually showed that the C-terminal region is crucial for asexual development, and the N-terminal region has an ancillary role. In addition, site-directed mutations of the predicted key catalytic residues in both regions yielded distinct loss of function phenotypes, indicating that both regions likely function as enzymes. Furthermore, the replacement of the N-terminal region by the bacterial homolog gene LSEI_0440 and the C-terminal region by the PA5508 gene resulted in the maintenance of functionality. The results, therefore confirm that both regions of FluG perform enzymatic functions in vivo. Our findings situate FluG as a putative bifunctional enzyme that may regulate the balance between vegetative growth and asexual development through the fine tuning of certain intracellular intermediates.

## BS06

# RbfA drives ribosome assembly by controlling the maturation state of the 30S decoding center

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Ribosome biogenesis is an essential process for the cell that requires the coordinated participation of a variety of protein assembly factors. In bacteria these factors include Era, RbfA, and RsgA, which play intertwined roles during the late assembly phase of the 30S subunit. Specifically, these factors assist in the folding of the central decoding region by ensuring the correct placement of the 3'minor domain (helices 44, 45 and the 3'16S rRNA end) between the rRNA domains forming the 30S head, body and platform. To further our understanding of the specific role played by RbfA in this process we have used NMR and cryo-EM to study the structure of RbfA in isolation and in complex with the 30S ribosomal subunit. Cryo-EM specifically revealed 3 distinct states of the 30S subunit (3.1-4.5 Å); the first a mature like conformation unbound by RbfA, the second a RbfA bound conformation characterized by an immature/disordered decoding center, and the third with an immature decoding center harboring an alternative structure for the rRNA linkers between h28, h44 and h45. Together these results show how the structure of RbfA adapts to bind the 30S subunit, at a site in the mRNA exit channel such that it displaces S21 while interacting with h28 and the 3'-end of the 16S rRNA. The interaction between the KH-domain of RbfA and the 3'-end of the 16S rRNA positions the later distinctly from its position in the mature 30S subunit, disrupting 16S rRNA interactions, and destabilizing the structure of the decoding center. These results are consistent with the idea that assembly factors, like RbfA, guide 30S assembly through distinct immature 30S conformations. Moreover, the role described here for RbfA is reminiscent of that proposed for the eukaryotic assembly factor Pno1 in 40S subunit assembly, indicating an underlying similar mechanism for small ribosome biogenesis.

## BS07

# Structural studies on novel translational inhibitors: toward the development of antibiotics with a new mechanism of action

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One of the main research lines undergoing in our group is toward the understanding of key molecular recognition events essential to antibiotic action as it offers opportunities to design inhibitors of protein synthesis for development of novel anti-infectives. The extensive use and abuse of the limited classes of antibiotics currently used in the clinical setting has led in fact to an exponential emergence of resistance mechanisms posing a serious threat to human health and urging the development of novel antimicrobial agents.

Among the studies recently conducted, Tigecycline (TIG), and GE81112 represent two different approaches that can be used to produce new antibiotics. TIG is the first representative of a third generation of derivatives based on the chemical scaffold of tetracyclines (TET). Using X-ray crystallography, we have determined the binding mode of TIG on the ribosome detailing the conformation of the C9-tail and its interactions with the ribosomal elements. Importantly, several pharmaceutical companies are developing a variety of additional C9 derivatives (i.e. TP-271, TP-6076, Omadacycline) that are in various stages of clinical trials opening possibilities for industrial collaborations.

GE81112 is a natural product composed of four non-proteinogenic amino acids and is a good example of a novel anti-infective more recently discovered. Although still in its early phase, it has the required characteristics for antibiotic development, namely GE81112 has (1) a novel chemical scaffold, (2) high bacterial selectivity and low/no toxicity toward eukaryotic cells, and (3) a rather broad spectrum of action towards several Gram-positive and Gram-negative bacteria. Our biochemical, X-ray crystallography and cryo-electron microscopy studies have revealed that GE81112 has a unique mechanism of action and binds to the ribosome in an essential and distinct pocket. Accordingly, the primary aim of this research is to capitalize on the extensive characterization of GE81112 and chemically synthesize this natural product and more potent derivatives guided by our structural and biochemical analysis.

## BS08

# Transfer of nucleoprotein complexes into human cells through bacterial Type IV Secretion Systems

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Bacteria utilize secretion systems to send specific proteins. Type IV Secretion Systems (T4SS) can translocate proteins, DNA, or nucleoprotein complexes, a capacity that makes them unique biologically. In addition, the fate of the substrates can be the extracellular milieu or another cell, either prokaryotic or eukaryotic, adding biomedical and biotechnological potential. T4SS play key roles in prokaryotic communication processes including horizontal DNA transfer by conjugation, or virulence by injection of effector proteins into eukaryotic cells, among others.

During bacterial conjugation, a nucleoprotein complex, formed by a protein known as relaxase linked to a DNA strand, is transferred through a T4SS into a recipient bacterium. Our group has been long interested in relaxase-driven DNA transfer into recipient prokaryotic and eukaryotic cells. We have reported DNA transfer to human cells through the T4SS of several human pathogens (1,2), and we are interested in deciphering the possible biological role of this phenomenon and exploiting its potential as a DNA delivery tool.

We also aim to characterize the molecular requirements for relaxase-DNA recruitment and the fate and activities of the nucleoprotein complex in the recipient cells. We previously showed that TrwC, the conjugative relaxase of plasmid R388, shows site-specific recombinase and integrase activities in the recipient, leading to integration of the incoming DNA into a target site present in the recipient genome (3). We analyzed the effect of TrwC on integration of the incoming DNA in the human genome, after translocation through the T4SS of *B. henselae*. We found that TrwC increases random integration efficiency by 2-3 logs (4). We propose that relaxases protect the incoming DNA, favoring its integration. Now we are trying to combine this system with site-specific nucleases, in order to obtain an in vivo DNA delivery system for site specific modification of the human genome.

In summary, we are pushing the limits of the modular capacity of conjugative relaxases and checking their functionality in prokaryotic and eukaryotic recipient cells. We hope to gain knowledge on the possible natural roles of these non-canonical substrate/T4SS pairs, and to develop genomic modification tools based on bacterial delivery of nucleoprotein complexes competent for integration into the human genome.

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## BS09

# Structural and Immunological Studies of Schmallenberg and Bovine Viral Diarrhea Viruses

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Infectious diseases represent the major causes of death worldwide. Some of these diseases are caused by viruses, entities that permeate the entire biosphere and infect organisms of the three domains of life: Bacteria, Archaea and Eukarya. With current climate change, global transportation and livestock trading, combating viral infection in humans and animals continues to be an ever-growing challenge to public health.

The Abrescia Lab - focuses on determining the molecular mechanisms governing viral pathogenesis using structural methods. Two viral targets of special importance for understanding the mechanisms of veterinary viral entry are:

**Schmallenberg virus** (SBV), a pathogen member of the Bunyaviridae family, has shown to induce widespread abortion storms in affected sheep and goats. The presence of SBV has already been demonstrated in southern France, as well as in the regions of Catalonia and Andalucia, thereby making an eventual outbreak in this area very likely.

Here, we present the work being performed in our laboratory which integrates immunological and structural studies. We demonstrate that DNA vaccine candidates, validated in small animal models, can induce a potent immune response. Furthermore, we will also discuss our current work in elucidating the high-resolution structure of SBV through cryo-Electron Tomography.

**Bovine Viral Diarrhea Virus 1** (BVDV) belongs to the Flaviviridae family, being one of the most important pathogens affecting bovines, causing respiratory, reproductive and gastrointestinal diseases and economical losses in farms. Although BVDV belongs to a different genus of Hepatitis C virus (Pestivirus and Hepacivirus resp.), both seem to present similarities in their structure (although pleomorphic), genome and mechanism of infection, in addition to evidences suggesting a similar fusion mechanism via the glycoproteins embedded in the lipid envelope. Since there is no 3D information about the virus morphology or a molecular framework for the virus-cell infection, here first we explore the interactions of glycoproteins Erns and E2 with specific antibodies, describing the spatial arrangement between the mAbs and corresponding glycoproteins.

# BS10

## Flavivirus as probes to study *Brucella* effector proteins

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*Brucella* spp. are facultative intracellular pathogens that are able regulate the inflammatory response and manipulate vesicular trafficking of cells, establishing a favorable growth niche for themselves. This process is accomplished through its Type 4 Secretion System (T4SS) and in the host side involves the endocytic pathway (early and late endosomes and lysosomes), the endoplasmic reticulum (ER) and autophagic vesicles. It is very likely that all these pathways are regulated by the action of several effector proteins secreted by *Brucella* T4SS. However, there are few effector proteins identify so far and all of them by different strategies and methods. So, it is reasonable to think that new strategies to detect *Brucella* effector proteins can identify new subsets of real effector proteins.

*Flaviviridae* are enveloped viruses that use specialized machinery to fuse viral and host cell membranes after internalization through the endosomal pathway. After internalization, they replicate and exit from the cell manipulating host membranes, mainly ER, Golgi and autophagic vesicles. This similarity in the ecological niche among *Brucella* y *Flaviviridae* made us hypothesize the presence in *Brucella* of bacterial effector proteins with the potential of interfering with the virus replication cycle. And therefore, the use of a new screening method based on this interference could reveal new *Brucella* effector proteins. We are using a novel interference system in which the ectopic expression of putative *Brucella* effector proteins takes place simultaneously to the infection of a flavivirus in the same cell. This interaction is tracked by flow cytometry, measuring the expression of the putative effector protein on one hand, and the fluorescence associated with the viral replication on the other. Changes in the virus fluorescent levels may identify new effector proteins that will be studied to determine their role in *Brucella* pathogenicity.

# BS11

## Genetics of bacterial biofilm formation process

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Our laboratory investigates fundamental mechanisms of regulation of bacterial biofilm development with a focus on human pathogens. We are interested in understanding how signal transduction systems control the transition between planktonic and sessile lifestyles. We also study the composition of the biofilm matrix and the function of these compounds during infection. In particular, we do research on basic principles of RNA overlapping transcription and its role in gene expression, on mechanisms of exopolysaccharide synthesis regulation and on how surface proteins of the biofilm matrix mediate intercellular adhesion and interactions with host cells. Very recently, we have deconstructed the two-component signal transduction system of *Staphylococcus aureus*. For that, we have generated fifteen *S. aureus* strains, each containing just a single TCS, as well as a mutant lacking all 15 non-essential TCSs. This powerful and unique genetic resource will allow us to unambiguously determine the contribution of each individual TCS to the regulation of peptidoglycan biosynthesis and antibiotic resistance.

# BS12

## Magnetotactic bacteria and magnetosomes: promising biomedical tools

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Magnetotactic bacteria (MTB) are a diverse group of aquatic microorganisms with the ability to orientate along the Earth's magnetic field lines to migrate and reach optimal conditions for their growth. This fascinating capacity is due to the presence of membrane-enclosed magnetic nanoparticles, called magnetosomes that are arranged into highly ordered chain-structures inside the bacteria. The physical and chemical characteristics of magnetosomes are under fine genetic control and, therefore, each species of magnetotactic bacteria synthetizes different nanoparticles in terms of morphology, size and composition. In our group, we have long experience working with *Magnetospirillum gryphiswaldense* MSR-1 (Figure 1A), which synthesizes cubo-octahedral shape magnetite ($Fe_3O_4$) nanoparticles with a mean diameter of 45 nm (Figure 1B). On the last few years, we have studied several aspects of this strain such as the biomineralization process for magnetosome synthesis [1] as well as the magnetic configuration and shape of the magnetosome chain [2], among others [3]. Therefore, now we aim to translate this expertise into two other species of magnetotactic bacteria: *Magnetovibrio blakemorei* MV1 and *Magnetococcus marinus* MC-1.

Many different applications have been proposed for both MTB and magnetosomes due to their unique physical properties. Particularly, we focus on the role that these agents may play in cancer therapy, as drug delivery carriers and as heating agents for hyperthermia treatment [4]. Thus, we study the cancer cell-magnetosome interaction, focusing on the internalization process and the effect in terms of cell viability. Moreover, we investigate the guiding of the bacteria with external magnetic fields. The ultimate goal will be to load MTB and/or magnetosomes with anticancer drugs and magnetically guide them to the tumour area where the drugs will be released. Once there, the application of an alternating magnetic field will make the magnetosomes to release heat, increasing the local temperature and debilitating the cancer cells without damaging other healthy tissues. Consequently, the effect would be localized minimizing side effects that conventional antitumoural techniques present.

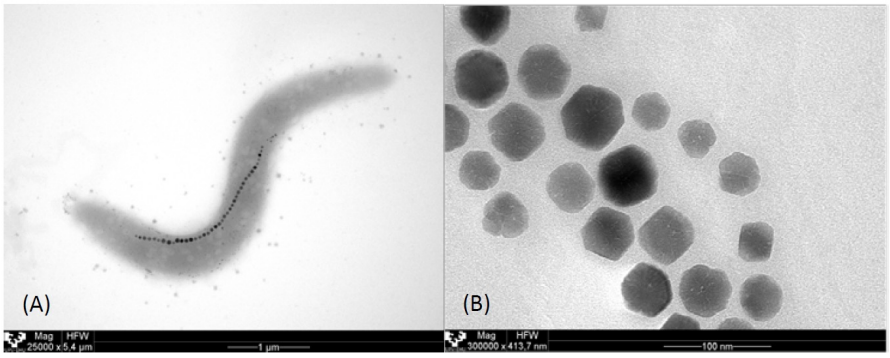


Figure 1. TEM images of (A) *Magnetospirillum gryphiswaldense* MSR-1 and (B) extracted magnetosomes

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## BS13

# Molecular mechanism for the subversion of the retromer coat by the *Legionella* effector RidL

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Deciphering microbial virulence mechanisms is of fundamental importance for the treatment of infectious diseases. *Legionella pneumophila*, the causative agent of Legionnaires' pneumonia, hijacks a variety of host cell factors during intracellular growth. Herein, we uncovered the molecular mechanism by which the *L. pneumophila* effector RidL targets the host VPS29, a scaffolding protein of endosome-associated sorting machineries. Using X-ray crystallography, we determined the structure of RidL, both alone and in complex with retromer. We found that RidL uses a hairpin loop similar to that present in cellular ligands to interact with retromer. This sophisticated molecular mimicry allows RidL to outcompete cellular ligands for retromer binding, explaining how *L. pneumophila* utilizes the endosomal sorting machinery to facilitate targeting of effector proteins.

# Topic 3:

## Environment & Food

# EF01

## Analysis of bacterial stress responses associated with climate change

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In the last decade, the detection of facultative pathogenic *Vibrio* strains and the incidence of *Vibrio*-associated diseases related to exposure and consumption of contaminated water have increased. Many researchers have suggested a causal link between global warming (and the subsequent rise in sea surface temperature) and enhanced spread of vibrios. In this context, the main goal of our group is focused on study of *Vibrio* responses to environmental changes related to ocean warming and the concomitant spread of diseases caused by pathogenic species.

We have used *Vibrio harveyi*, a marine bacterium, as a model organism to study physiological, morphological and gene expression changes involved in the temperature-dependent adaptation of *Vibrio* species in seawater microcosms. This study will be expanded in the coming years by analyzing the effects of additional stress factors (e.g. salinity and solar radiation), as well as those caused by their combined action along with temperature. Particular attention will be given to discovery, validation and functional analysis of small regulatory RNAs (sRNAs) playing essential regulatory role in bacterial adaptation to stress by using *Vibrio harveyi* as model organism.

Given the recent spread of invasive pathogenic species, including those of the genus *Vibrio*, one of our research lines is aimed at developing new tools enabling to monitor the seasonal presence of *Vibrio* species by sampling and analyzing seawater in different areas of the Biscay Bay. Moreover, the survival model of *Vibrio* spp. and the potential to elicit infections will be assessed by using a host-pathogen model system, in which the host will be a mussel species widely used in the Basque coast and worldwide as an environmental biomarker.

## EF02

# Inhibitors of bacterial conjugation, new strategies for the control of antibiotic resistance in the environment

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The increase of infections caused by multiresistant pathogenic bacteria is one of the major problems of the public health system. But although the ultimate consequences of this situation challenge us with a medical problem of the first magnitude, the presence of antibiotics in urban and agro-livestock environments, aquifers, and wastewater contributes to increase the environmental pressure on bacteria to maintain and disseminate antibiotic resistance genes (ARG) by mobile genetic elements (MGE), increasing their number in the human microbiota, in the clinical environment where infections by multiresistant bacteria are a growing concern, in agricultural and livestock spots, and in the environment in general. Therefore, novel strategies are needed to address this problem from an environmental perspective, acting in the different ecosystems where the resistance genes are selected and disseminated.

An action proposal could be based on the inhibition of bacterial conjugation since it is the main mechanism of dissemination of ARG among bacteria. In particular, coupling proteins (T4CP), which are present in all conjugative systems and are essential for bacterial conjugation to take place, represent a good target for the search for bacterial conjugation inhibitors. Consequently, our hypothesis proposes that specific inhibitors of T4CP will control the spread of resistance among bacteria and, therefore, the emergence of multiresistant pathogenic strains.

## EF03

# Marine Microbiology through molecular lens: From ecology to applications

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At the Molecular Ecology and Biotechnology Group of AZTI, we use molecular approaches to understand marine ecosystems and improve management of their resources, including the study of the diversity and functional roles of marine microorganisms in the environment. We combine metabarcoding, Omics (genomics, metagenomics, metatranscriptomics), and more classical microbiology approaches (cell cultures, experimentation) in order to unveil the diversity of microbes that can be found in the marine environments (seawater, sediments), their functional roles, how environmental or human-induced stressors influence their activity (ocean warming, pollution) and potential biotechnological applications (e.g., omega-3 production).

## EF04

# Metagenomics in beekeeping, viticulture and animal food production

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Genomic Resources uses the advances in Next Generation Sequencing for the taxonomic and functional characterization of the microbial communities in ecosystems related to 1) beekeeping, 2) viticulture and 3) animal food production. The common principle that guides our projects is that with a greater knowledge on the microorganisms-host interactions, these sectors could develop strategies for their modulation and /or manipulation that could entail a decrease in disease impact, the reduction of the number of chemical treatments and their accumulation in the environment, and an improvement of the quantity, quality and safety of the produced food.

Regarding beekeeping, the projects we are involved in try to elucidate the correlation between the microbiome of the hive and its tolerance to the pathogen Varroa destructor. The ultimate goal is to identify microorganisms that could be used to reinforce the health of the hive against varroasis disease. Those microorganisms will be good candidates as a target for probiotic development, that could be used as an alternative strategy to the actual indiscriminate use of chemical treatments against varroasis.

Regarding viticulture, our projects aim to characterize soil and grape microbial profiles to determine the health and microbial terroir of vineyards. In addition, we intend to advance in the search of preventive strategies against *Botrytis cinerea* pathogen, to provide viticulturist essential information that would lead to i) minimize losses in production and grape quality associated with botrytis disease, ii) limit fungicides treatment and iii) ensure wines of higher quality.

Finally, we pretend to provide insight into the effects of the biomolecular interactions between feed additives, gut microorganisms and host animals in feed and food production by implementing a novel holistic framework, the holo-omic framework, to increase the efficiency of feed additives, as well as improving final food products and food safety.

## EF05

# Presentation of the SOIL MICROBIAL ECOLOGY GROUP from NEIKER - Basque Institute of Agricultural Research and Development

*Carlos Garbisu, Mikel Anza, Fernando Blanco, Lur Epelde, June Hidalgo, Leire Jauregi, Oihane Salazar and Julen Urra*

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The SOIL MICROBIAL ECOLOGY GROUP at NEIKER-Tecnalia (www.soilmicrobialecology.com) is most interested in the impact of different sources of environmental stress (contamination, agricultural practices, climate change) on soil health. Our research is focused on the utilization of soil microbial properties (mainly, those related to the biomass, activity and structural/functional diversity of soil microbial communities) as biological indicators of (i) the impact of disturbances on soil health and (ii) the efficiency of remediation methods (bioremediation and phytoremediation of contaminated soils). Regarding agricultural practices, we work on the utilization of soil microbial properties as biomonitoring tools for the assessment of the improvement in soil health derived from the application of environmentally-friendly practices (e.g., no-tillage, organic fertilization). Finally, an important part of our research is nowadays focused on the risk of dissemination of emerging contaminants (e.g., antibiotics and antibiotic resistance genes) in agricultural soils and crops.

We determine a great variety of parameters as indicators of soil microbial (i) BIOMASS: biomass carbon, ATP, DNA, ergosterol, glomalin, substrate-induced respiration, total bacteria, total fungi, total archaea, gene abundance by qPCR/high-throughput qPCR/droplet digital PCR, etc.; (ii) ACTIVITY: basal respiration, potentially mineralizable N, nitrification potential rate, enzyme activities (β-glucosidase, β-glucosaminidase, cellulose, chitinase, xylosidase, urease, arginine deaminase, amidase, protease, L-leucine aminopeptidase, L-alanine aminopeptidase, arylsulfatase, acid and alkaline phosphatase, dehydrogenase, FDA-fluorescein diacetate hydrolysis, gyrase), etc.; and (iii) DIVERSITY: community-level genetic profiles with ARISA and DGGE, community-level physiological profiles with Biolog™ plates, functional microarrays, high-throughput sequencing (16S rRNA/18S rRNA/ITS metabarcoding and shotgun), etc. In addition, we quantify functional genes (biodegradation, nutrient cycling, antibiotic resistance, horizontal gene transfer) and ecosystem attributes (resistance, resilience, suppresiveness, etc.). Last but not least, we study the traits of plant growth-promoting rhizobacteria and bacterial endophytes (ACC deaminase activity, siderophore production, phosphate solubilisation, indolacetic production, etc.) and perform soil ecotoxicity tests (Microtox, MARA, LumiMARA, PICT, MIC values, etc.).

## EF06

# Gut Microbiomics and Membrane Lipidomics as molecular approach for precision nutrition

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Food, as a potent external stimulus on individuals, has an impact at all cell levels and thus at our tissues and organ functioning. Differences in the response of people to dietary components have been well documented and this provides the basis, and motivation for developing personalised nutrition strategies.

The overall goal of precision nutrition is to preserve or improve health status using genetic, phenotypic, medical, and other relevant information about individuals to adapt the nutritional recommendations to their individual needs and preferences. To this end, precision nutrition approaches include, in addition to genetics, other factors such as dietary habits, food behaviour, physical activity, the gut microbiome and the metabolome.

Despite the progress of the OMIC sciences (genomics, proteomics, lipidomics, etc.) as diagnostic tools, there are not yet resolved intervention strategies integrating all the information to provide diet solutions.

Following this approach, Azti has made a strategic bet to apply membrane lipidomics together with gut microbiome analysis for personalized food design and for providing accurate nutrition to prevent and control obesity. The use of mature red blood cell (RBC) membranes as representative site for all other body tissues, is an established protocol for membrane-based molecular diagnostics. Emerging evidence suggests that some of the effects of dietary lipids on host metabolism may be mediated by modifications of the gut microbiota composition, which result in altering metabolic properties of the individual.

AZTI has carried out an observational study in Biscay with 100 healthy children between 6 to 16 years, to monitor RBC membrane lipid profile and gut microbiome. The aim is to elucidate the type of relationship between diet and metabolism of the participants. The observed changes in the identified key molecular parameters will serve to establish precisely the nutritional requirements which will lead to new food formulations and effective supplements to prevent child obesity.

# EF07

## Molecular epidemiology of *Campylobacter* and *Arcobacter*

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The genera *Campylobacter* and *Arcobacter* are members of the family Campylobacteriaceae. Our group has been working for years in the study of campylobacteria as food and waterborne pathogens. Nowadays, and due to the great importance it has gained in recent years, we are mainly focused on the study of *Arcobacter*; indeed, members of this genus can act as potential emerging pathogens and / or as zoonotic agents.

Food Security policies should be based on risk analysis. The identification of those potentially harmful agents (biological, chemical and physical) that may be present in a particular food or group of foods is the first stage of this analysis (AECOSAN, 2013), and is particularly important when dealing with emerging pathogens like *Arcobacter* (ICMSF, 2002). This genus is associated with human and animal disease (Ramees et al., 2017) but its pathogenicity and virulence mechanisms are still unclear. Our main objective is to identify the virulence factors of *Arcobacter*, those implied in human illness.

1st research line. Genotying of *Arcobacter* by MLST aiming both, to characterize the *Arcobacter* species and strains isolated food products and water, and to establish possible phylogenetic relationships.

2nd research line. Virulence factors of *Arcobacter*, aiming to determine the prevalence of the virulence associated genes described for *Arcobacter* in food and water derived isolates, together with the virulence phenotype (biofilm formation ability, capacity of adhere and/or invade cells and haemolysis, among others) of those isolates.

3rd research line. Functional analysis of genes, by reverse genetic analysis, aiming to determine the function of the virulence associated genes of *Arcobacter*.

4th research line (recently implemented line). Characterization of human clinical isolates, aiming mainly to establish a collaboration in studies of clinical and epidemiological interest between the Clinical Microbiology Service of the University Hospital of Álava and our research group. Currently we do not only collaborate with *Arcobacter* isolates derived from human faeces, but also with *Pseudomonas aeruginosa* isolates derived from lower tract respiratory infections.

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# EF08

## Molecular microbiology in environment, food and clinical settings

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Our research group has traditionally focused its activity on the study of diverse microorganism with clinical, food and environmental interest, using culture-based traditional techniques and also molecular and sequence-based techniques for our research. In this sense, we are working on different research lines, being one of them the study of the epidemiology, genomic and developing of new detection techniques of Salmonella and other foodborne bacteria. Salmonella is one of the leading causes of gastroenteric diseases, and typhoid fevers worldwide, mainly caused by products and derivatives of animal origin and water. Besides, new pathogenic variants of *Salmonella* have emerged in recent years, so a rapid and effective detection of the bacteria is absolutely necessary. Among years, we have developed different Salmonella typing methods and rapid detection techniques from food samples, like real time PCR and multiplex PCR based methods. In the latest years, we have focused the research on Salmonella epidemiological study using whole genome sequencing and sequencing-based typing techniques (MLVA, MLST). Using these methods, we are trying to study the evolution and pathogenicity factors of *Salmonella enterica* monofasic variants and *Salmonella enterica* serovar Typhi strains.

On the other hand, we are now working on the characterization and epidemiological study of *Neisseria gonorrhoeae* strains that have been isolated in Álava in the last years. The gonococic infection is increasing worldwide, but also its antimicrobial resistance, becoming a world health problem. Nowadays the information about the *N. gonorrhoeae* strains isolated in Álava is not enough, so our aim is to characterize those strains and to know the level of antimicrobial resistance among those strains. Among our objectives is to find antimicrobial resistance determinants in strains genomes sequences and associate them to the resistance observed in vitro in the laboratory.

Finally, our newest research line tries to characterize the microbial biodiversity of Álava region water sources. For this purpose, different microbiological traditional techniques, whole genome sequencing and metagenomics approach are used. Data obtained in the metagenomic study of Salburua wetland water microbial community composition showed us that it is a high richness ecosystem. Now, characterization of bacteria and archaea community of Salinas de Añana saltern water is our lastest objective. The halophilic characteristic of Salinas de Añana valley makes it an extraordinary scenario for the development of a microbial world living in extreme conditions. In this sense, the purpose of our work is to go deep at the microbial characterization of the salty waters for a better knowledge of the genera and species present in this ecosystem and, if possible, to identify and isolate bacteria with biotechnological applications and with a clear antimicrobial activity.

## EF09

# Selection and improvement of microbial starters for the fermentation industry

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The employment of starter cultures for the fermentation industry has become a very common practice the guarantee the production of high-quality food with consistent characteristics. Microorganisms with good technological properties, providing sensory desirable compounds and lacking the ability to promote metabolites of safety concern to humans have been the main focus of our research studies.

Specifically, we have been studying the indigenous population of lactic acid bacteria from oenological fermentations in the Rioja Alavesa region in order to develop specific starter cultures to produce safe wines with superior characteristics. In that way, the aim of the study was to identify indigenous strains lacking the ability to produce biogenic amines and ethyl carbamate precursors and which own suitable technical and organoleptic characteristics. A great variety of *Oenococcus oeni* genotypes have been identified and characterised so far in musts and wine samples from several wineries. None of the isolated strains produced biogenic amines; however, some strains could degrade the amino acid arginine, highlighting the potential production of precursors of ethyl carbamate, a probable carcinogen for humans. Selected strains which were unable to produce those metabolites will be further tested for their technological and organoleptic properties to perform a final selection of the best suited for the production of starter cultures for the wine industry.

We have also been studying the development of yeast starter cultures applicable to brewing fermentations able to reduce the concentration of mycotoxins (specifically Ochratoxin A) in the final product. In this case a genetic improvement strategy was employed in which hybrids yeast bearing on the one hand positive technological and organoleptic characteristics and on the other the ability to degrade ochratoxin A were constructed. This technique allowed to obtain improved strains that are not considered genetically modified organisms and can therefore be employed as starter cultures in the brewing industry. The yeast developed could reduce ochratoxin A up to 50% of the original concentration and produced beer with distinctive organoleptic properties.

Our work is currently focused on the oenological and brewing sector, but it sets the basis for developing starter cultures for all types of fermented foods.

## EF10

# Strategies to improve heterologous synthesis of docosahexaenoic acid in *Escherichia coli*

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Some marine bacteria, such as *Moritella marina*, are able to produce certain amounts of the nutraceutical docosahexaenoic acid (DHA) thanks to a specific enzymatic complex called Pfa synthase. Moreover, *Escherichia coli* heterologously expressing this *pfa* gene cluster from *M. marina* is also able to produce DHA. The aim of this study was to find genetic or metabolic conditions to improve DHA production in *E. coli* or any other microorganism. Firstly, we studied native promoter expression pattern in *E. coli* and replaced these promoters by inducible pBAD system in order to increase the production of the final product. Secondly, we altered the canonical carbon flux in *E. coli* to improve the availability of substrates for Pfa complex by two strategies: using the exogenous compound cerulenin, and deleting the main initiation enzyme of a competing pathway. Both strategies exploit a substrate competition mechanism between the native fatty acid synthase from *E. coli* and the heterologous Pfa complex from *M. marina*. Finally, we improved *E. coli* growth at low temperature by introducing two psychrophilic chaperonins, Cpn10 and Cpn60 from *Oleispira antarctica*, in order to improve protein folding. These approaches have increased the overall efficiency of this process, and could be used for biotechnological optimization in different organisms to synthesize DHA or other polyunsaturated fatty acids.

## EF11

# Bacteriophage biocontrol of foodborne pathogens and food spoilage bacteria

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The use of bacteriophages (phages) and their lytic proteins as natural biocontrol agents to reduce the prevalence of food-borne pathogens and food spoilage bacteria within the farm-to-fork process is being increasingly accepted.

Phages are viruses that specifically infect and kill bacteria, being widely distributed in the environment and often consumed in our diet as they are present in the natural micro flora of several food products. The use of phages as biocontrol technology is desirable as they are specific towards the target pathogenic / spoilage bacteria, they are harmless to humans, animals and plants, and they do not affect the normal microflora of foods nor alter attractive organoleptic food properties.

Work conducted by our group on the use of phages and purified phage lytic proteins as food safety strategy is mainly focused on two food-borne pathogens of major concern nowadays: *Campylobacter* spp. and *Listeria monocytogenes*. In addition, the use of phages as natural bio-preservatives towards different food spoilage bacteria is also being studied.

In this scenario, we have isolated more than 300 lytic bacteriophages with specific activity against target pathogenic and spoilage bacteria, including antibiotic-resistant strains. Current research is mainly focused on the in-depth characterization of the most promising phages of our large collection by considering the most relevant safety and technological properties for industrial applications and large-scale commercial production. The final objectives of our research line are to design and develop innovative phage-based products to control target pathogenic or spoilage bacteria in food products and food processing environments, and, to support near future EU regulation on safety assessment and efficacy of phages application.

## EF12

# Biological remediation of contaminated soils aimed at soil health improvement

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The survival and well-being of our society are inextricably linked to soil health and fertility. Healthy soils can: (i) store, filter and transform water and nutrients; (ii) produce food and fibre; (iii) host biodiversity; (iv) regulate the climate; (v) decompose organic matter; (vi) detoxify contaminants; etc. Regrettably, in the last decades, soil contamination has become a huge environmental problem that is, at present, seriously affecting the health and functional sustainability of our soils. Phytoremediation with contaminant-tolerant plants and bioremediation with degrading microorganisms are both considered cost-effective, environmentally-friendly, socially-accepted biotechnologies of great potential for the sustainable remediation of contaminated soils. It must be highlighted that the ultimate goal of these biological remediation technologies (actually, of any remediation technology) must be not only to remove the contaminants from the soil but, most importantly, to recover soil health, i.e. the capacity of the soil to sustainably carry out its ecological processes, functions and ecosystem services. Soil microbial parameters, particularly those reflecting the biomass, activity and diversity of soil microbial communities, have great potential as biological indicators of soil health. The main objective of our work is to assess the efficiency of different biological remediation (i.e., phytoremediation, bioremediation) strategies through the determination of a variety of soil microbial properties with potential as biological indicators of soil functioning. In particular, our results indicate that the presence of toxic heavy metals frequently has a considerable adverse impact on soil microorganisms and, hence, soil health. The negative effects of inorganic and/or organic contaminants on soil health can be partially or totally recovered through the application of phytoremediation (phytoextraction, phytostabilization) and bioremediation (biostimulation, bioaugmentation) practices. The sensitivity, rapid response and integrative character of soil microbial properties make them invaluable biomonitoring tools for the assessment of the efficiency of biological remediation processes.

# EF013

## Marine microbes research group: looking for answers in the sea

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Our research group studies the diversity, functional activity and interactions of the prokaryotic and bacterivorous protist communities in the marine environment. We use several molecular methods of genome analysis to investigate the structure, the composition and the diversity of the bacterial communities. Likewise, in order to establish links between function and diversity, we compare this information with the measurements of distinct physiological activities carried out by those bacterial communities that are related to the transformation of marine organic material, such as the consumption of organic compounds, the growth and the respiration rates. We are also interested in other fields of study such as the analysis of the chemical composition and the transformation of organic matter in seawater, as well as the study of the interrelationships and chemical communication that occur between bacterivorous protists and their bacterial prey.

In order to carry out our experiments, we regularly collect samples of seawater at a station located in the coast of Armintza (Bizkaia) in the Eastern Cantabrian Sea, and we have also participate in oceanography cruises around the world. Although we mainly focus our research on surface waters, we have also analysed deep waters of Atlantic, Indian and Pacific oceans in the context of the research project "Circunnavigation Expedition Malaspina 2010". As marine bacterioplankton is the major biotic component of the oceans generating carbon dioxide, the most important greenhouse gas, in this expedition we assessed the impact of the global climate change on the ocean by analysing the hydrolytic enzymatic activities of the bacterioplankton at different temperatures through the water column.

The results obtained in Expedition Malaspina indicated the need to acquire predictive power on the functioning of extracellular enzymes in future scenarios of global warming. These enzymes are produced and released by bacterial communities driven by the environmental characteristics of the ecosystem, and therefore the aims of our current research project “ENZIMA” are: i) to determine the temperature sensitivity of the kinetic parameters of a set of model extracellular enzymatic activities, which are responsible of the hydrolysis of organic compounds rich in carbon, nitrogen and phosphorus in coastal waters, ii) to analyse how the affinity of the produced enzymes can regulate the final hydrolytic activity, iii) to assess the possible disturbances in the C:N, C:P and N:P stoichiometric ratios of the generated organic matter available for bacterial remineralization, and iv) to identify links between the enzymatic activities and fundamental properties of the ecosystem, such as seawater temperature, the composition of the bacterial community, primary producers, inorganic nutrients and dissolved organic matter.

This project is innovative because no studies on this research topic have been carried out in coastal waters, one of the four major biomes or oceanic domains. Furthermore, the results obtained in ENZIMA will provide tools for predicting the behavior of microorganisms, from a biogeochemical point of view, in future scenarios of global warming. The results will also

contribute to a better understanding of factors affecting the composition of the bacterial communities throughout the seasonal cycle and provide valuable information about the relationship between the diversity and the functioning of microorganisms in a marine coastal ecosystem.

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