PROGRAMA DE DOCTORADO EN MICROBIOLOGÍA

JORNADA CIENTÍFICA 2025/26

26 de mayo de 2026

SALA DE GRADOS DEL EDIFICIO DE BIOLOGÍA.
UNIVERSIDAD AUTÓNOMA DE MADRID (UAM)

Programa de la Jornada

09.45 Samuel Cirés Gómez. Apertura de la Jornada.

Sesión I. Microbiología Ambiental y Fisiología Microbiana.

Moderadores: Irma Marín Palma y Miguel Ángel Rodríguez Gabriel.

10.00. Sofía Galbán Méndez. Vertical profiling of airborne microbial communities across the atmospheric boundary layer.

10.15. Albano Díez Chiappe. Toxic cyanobacteria in Mediterranean National Parks: towards a better understanding of diversity, distribution, and emerging risks in freshwater ecosystems.

10.30. Víctor Ignacio Muñoz Hisado. Ecología microbiana de cuevas heladas de los Pirineos.

10.45. Esther Velasco Domínguez. Sulfur and Oxygen isotopes variation as a record of biological and inorganic processes at the Mars Analogue of Rio Tinto, SW of Spain, and its implications on Mars Sample Return Missions.

11.00. Descanso

Sesión II. Microbiología Clínica-Patógenos y Biotecnología Microbiana. Moderadores: Daniel López Serrano y Jesús Mingorance Cruz.

11.30. Laura Blas Muñoz. Discovering KREDs in natural diversity using microfluidics.

11.45. Carlos Murguiondo Delgado. Ingeniería computacional de la cutinasa de *Fusarium solani* para la degradación eficiente de poliésteres.

12.00. Ana Carlón Irala. Peste des Petits Ruminants Virus Disarms TRIM25 to Evade Antiviral Immunity.

12.15. Celia Alonso Gil de Gómez. Contribución del segmento M del ARN de la cepa 40Fp8 del virus de la fiebre del Valle del Rift a la atenuación viral.

12.30. Patricia Sánchez-Mora. Detección y caracterización genética del virus de la fiebre hemorrágica de Crimea-Congo en garrapatas del oeste de España (2017, 2020-2024).

12.45. Clausura.

Vertical profiling of airborne microbial communities across the atmospheric boundary layer

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Aerobiological studies have traditionally focused on near-surface sampling and horizontal biogeographic patterns, while vertical microbial structuring within the atmospheric boundary layer (ABL) remains poorly characterized. This knowledge gap is largely due to logistical constraints, including limited accessibility, the need for aerial platforms, technological challenges in collecting sufficient biomass over short sampling periods, and extreme meteorological conditions.

Here, we present an integrated approach to investigate airborne microbial communities across different levels of the ABL in a coastal Antarctic environment. Microbial samples were collected simultaneously at lower and higher atmospheric levels using aerial and ground-based platforms, and microbial community composition, abundance and morphometry were analysed using metabarcoding and microscopy-based techniques, respectively. The study was conducted at a low-orography coastal site in Antarctic Peninsula, representative of air masses from the Southern Ocean, and supported by atmospheric observations and air-mass trajectory analyses.

Our results reveal a clear vertical differentiation in airborne microbial communities. Communities sampled at higher atmospheric levels showed reduced diversity and distinct taxonomic signatures compared to those closer to the surface, consistent with selective processes acting during vertical transport and atmospheric residence. Communities from near-surface samples were comparatively more homogeneous, reflecting strong mixing of local biological sources, whereas communities from higher-altitude samples exhibited greater variability among the samples, influenced by broader-scale atmospheric transport. Despite these differences, partial overlap in community composition between atmospheric layers suggests vertical connectivity within the ABL. Variations in microbial abundance and morphometric cell characteristics further support the role of atmospheric structure and stability in shaping airborne microbial assemblages.

Acknowledgements: This work was supported by the Spanish Agencia Estatal de Investigación (AEI), Grant PID2020-116520RB-I00. Sofía Galbán was supported by a PIPF-contract fellowship (PIPF-2022/ECO-25833) from Comunidad Autónoma de Madrid government's (Spain). This work was supported by PEJ-contract fellowship (PEJ-2024-AI/COM-32565) financed by Comunidad Autónoma de Madrid government's (Spain) and co-financed by European Union. The authors are grateful to Unidad de Tecnología Marina (UTM-CSIC) and crews of the BIO Hespérides (Spanish Navy) for the logistic support in Antarctic campaigns. The authors acknowledge the computer resources, technical expertise and assistance provide by the Centro de Computación Científica at the Universidad Autónoma de Madrid (CCC-UAM) and the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model. The authors thank Luis Piñuel and José Antonio López Orozco from Universidad Complutense de Madrid (UCM) for their support in the collection and management of the meteorological data. Special thanks for Hilo Moreno for valuable recommendations and assistance during field sampling.

Toxic cyanobacteria in Mediterranean National Parks: towards a better understanding of diversity, distribution, and emerging risks in freshwater ecosystems

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Toxic cyanobacteria can adversely affect a wide range of organisms, including humans and animals, making their study particularly relevant in ecologically valuable protected areas.

This doctoral thesis addresses the proliferation of cyanotoxin-producing cyanobacteria in Monfragüe and Sierra de Guadarrama National Parks, encompassing diverse aquatic systems representative of the Mediterranean region. By combining sampling across reservoirs, ponds, streams, and mountain rivers with complementary methodologies such as genetic analyses, metabarcoding sequencing, cyanotoxin quantification and microscopy, this work aims to provide a comprehensive study of these events and to assess their potential risks.

In the reservoirs of Monfragüe, dense cyanobacterial proliferations containing concerning levels of hepatotoxins (microcystins) were detected, together with low concentrations of neurotoxins (anatoxin-a and saxitoxins). Comprehensive metabarcoding sequencing identified the toxigenic species and revealed unexpected highly heterogeneous communities in three studied reservoirs, despite their spatial proximity and hydrological interconnection, with water conductivity potentially influencing communities composition. Additionally, this study is the first report of the tropical-related species *Planktothrix spiroides* in Europe.

Extending the study to smaller waterbodies in Monfragüe (ponds and temporary streams) revealed the widespread distribution of toxic planktonic and benthic species. Variable degrees of fine-scale genetic overlap were detected among the different studied waterbodies. The combined methodological approach identified microcystin-producing *Microcystis* as the key toxic taxa and revealed the sharing of toxic genetic variants among hydrologically unconnected systems. These findings suggest a potential scenario of cyanobacterial connectivity across this protected landscape.

In rivers from Guadarrama, the results confirmed widespread, abundant and recurrent proliferations of the benthic neurotoxin-producing *Microcoleus*. Metabarcoding sequencing revealed a marked differentiation of *Microcoleus* genotypes between two adjacent rivers, suggesting the influence of environmental factors in shaping benthic assemblages. Toxic *Microcoleus* represents an emerging global concern and this study constitutes the first report of its abundant proliferation in Spain, expanding currently limited knowledge in Southern Europe.

Ecología microbiana de cuevas heladas de los Pirineos

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La criosfera engloba una amplia diversidad de ecosistemas caracterizados por la presencia de hielo. Aunque el interés por los microorganismos que habitan estos ambientes ha crecido notablemente, algunos sistemas criosféricos, como las cuevas heladas, continúan siendo escasamente estudiados. Estas cavidades representan archivos paleoclimáticos excepcionales, ya que permiten la conservación de hielo, protegiéndolo de la radiación solar, de las fluctuaciones térmicas y de contaminantes externos. Como resultado, albergan hielo perenne cuya antigüedad puede alcanzar decenas de miles de años. Este hielo es un registro químico, ambiental y biológico, capaz de conservar comunidades microbianas expuestas a condiciones extremas de baja temperatura y limitada disponibilidad de nutrientes.

Los objetivos de esta tesis han sido caracterizar las comunidades microbianas, tanto procariotas como eucariotas, presentes en cuatro cuevas heladas de los Pirineos (Devaux, Sarrios, Somola y A294). Para ello, se analizó su diversidad taxonómica, su interacción con la composición química de las cuevas, su respuesta frente al estrés térmico y su papel potencial en procesos biogeoquímicos, como la biomineralización.

Los resultados muestran que, aunque las cuevas heladas pirenaicas presentan una composición taxonómica comparable a la de otros sistemas geológicamente equivalentes, cada cueva alberga comunidades microbianas significativamente distintas. El tipo de muestra y su composición química resultaron factores clave para explicar los patrones observados. Asimismo, se demostró que estas comunidades pueden permanecer activas y responder a incrementos de temperatura. En una de las cuevas se identificaron minerales con morfologías compatibles con procesos de biomineralización. La reconstrucción de las rutas metabólicas sugiere que las comunidades microbianas presentes son potencialmente compatibles con dichos procesos, subrayando su estrecha relación con los procesos biogeoquímicos.

Las cuevas heladas representan ambientes extremos en los que las bacterias desempeñan un papel central. En un contexto de calentamiento global que amenaza este tipo de sistemas, su estudio resulta especialmente relevante para campos como la biotecnología y la salud pública. Esta tesis constituye una aproximación inicial a estos ecosistemas y pone de relieve la necesidad de investigarlos con mayor profundidad.

Sulfur and Oxygen isotopes variation as a record of biological and inorganic processes at the Mars Analogue of Rio Tinto, SW of Spain, and its implications on Mars Sample Return Missions

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The development of geochemical proxies for detecting possible early life on Mars is crucial for future space missions, particularly those aimed at returning samples to Earth. This study proposes an integrated mineralogical, chemical, and stable isotope approach using materials from Rio Tinto as a terrestrial analogue of Martian environments. Iron-rich sulfate minerals such as jarosite, which are present on Mars, make sulfur isotopes a promising tool for identifying potential biosignatures.

Mars' geological evolution is defined by three major geochemical environments, Phyllosian, Teiikian and Siderikian [1], each associated with distinct mineral assemblages. The Phyllosian environment formed under circumneutral conditions and is dominated by clays and phyllosilicates. The Teiikian environment reflects acidic conditions and is characterized by iron-rich sulfates, while the Siderikian environment represents dry, oxidizing conditions with anhydrous ferric oxides.

Rio Tinto's extreme geochemistry results from the weathering of pyrite-rich ores and the microbial metabolism of iron and sulfur compounds [2]. These processes lead to the formation of minerals such as jarosite, goethite, and hematite, which have also been observed in Martian deposits [1, 3]. Water and sediment samples collected along the river, from source to estuary, were analyzed for sulfur and oxygen isotopes. Sulfidogenic flow-through reactor experiments were conducted using sediments from both the upper reaches of the river and the estuary. The results show complex fractionation patterns, with decreasing $\delta^{34}S$ and increasing $\delta^{18}O$ values downstream.

No significant differences were observed between experimental reactors under varying pH conditions, although sediment buffering effects were detected. Mineral analyses indicate that jarosite and pyrite are isotopically consistent with dissolved sulfates in acidic zones. In the estuary, lighter sulfur isotope signatures in pyrite suggest possible biotic sulfate reduction.

Overall, the findings indicate that under acidic, Mars-like conditions, sulfur isotopes mainly reflect geochemical processes. However, in circumneutral environments, reduced sulfide minerals may preserve potential biosignatures, offering valuable targets for future Mars exploration.

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Discovering KREDs in natural diversity using microfluidics

Laura Blas Muñoz

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The discovery of novel biocatalysts is essential for the development of more sustainable and efficient chemical processes. Ketoreductases (KREDs) are particularly valuable due to their ability to catalyse stereoselective reductions of carbonyl compounds, which are key transformations in the synthesis of pharmaceuticals and fine chemicals. However, a large fraction of enzymatic diversity remains unexplored, especially in uncultured microorganisms. This PhD project aims to access this diversity through the development of high-throughput methodologies for the identification and characterization of new KREDs from metagenomic sources, combining microfluidic screening with biochemical and computational approaches.

A soil-derived metagenomic library of approximately 1.5×10^6 clones was constructed and used to establish a droplet-based microfluidic screening platform. Individual clones were compartmentalized in water-in-oil droplets together with a fluorogenic substrate, enabling the detection of enzymatic activity via fluorescence. Following multiple rounds of fluorescence-activated cell sorting (FACS) and enrichment, several candidates with putative KRED activity were identified and validated, leading to a reduced set of enzymes selected for further characterization.

Functional and computational analyses revealed that most of these sequences do not cluster with known KRED families, suggesting potential novelty. The corresponding genes were synthesized, expressed in *Escherichia coli*, and evaluated against a panel of alcohol substrates using NAD^+ and $NADP^+$ as cofactors. While several enzymes were obtained in soluble form, others required optimization strategies such as chaperone co-expression, highlighting limitations associated with heterologous expression in cellular systems.

To overcome these limitations, an in vitro ultra-high-throughput screening platform based on single-molecule compartmentalization in microfluidic droplets was developed. This approach eliminates dependence on cellular expression, enabling the exploration of a broader sequence space and facilitating the detection of enzymes that may be poorly expressed or toxic *in vivo*. The system integrates in vitro transcription–translation with DNA amplification to enhance sensitivity. Key parameters, including monoclonality, incubation time, and DNA recovery after sorting, were optimized, achieving a detection limit of approximately 200 droplets.

Current work focuses on improving DNA recovery and applying this in vitro system to new environmental metagenomic libraries.

Ingeniería computacional de la cutinasa de *Fusarium solani* para la degradación eficiente de poliésteres

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La degradación enzimática de bioplásticos como el ácido poliláctico (PLA) y el tereftalato de polietileno (PET) representa una alternativa prometedora para su reciclaje sostenible. Las cutinasas, hidrolasas capaces de hidrolizar enlaces éster en poliésteres naturales y sintéticos, han emergido como biocatalizadores de gran interés. Sin embargo, su aplicación industrial está limitada por su estabilidad y eficiencia catalítica, especialmente a temperaturas cercanas o superiores a la de transición vítrea (T_g) de los polímeros. En este trabajo se aborda la mejora de la cutinasa de *Fusarium solani* (FsC) mediante un enfoque combinado de diseño computacional y validación experimental en dos etapas complementarias.

En una primera fase, se emplearon las herramientas SCANEER [1] y FireProt [2] para diseñar variantes con mejoras en actividad y termoestabilidad. Las mutantes resultantes mostraron un rendimiento superior al de la enzima nativa, destacando FsC-fp por su elevada estabilidad térmica y su mayor eficiencia en la hidrólisis de PLA y PET. Estos resultados demuestran la capacidad de la ingeniería computacional para identificar mutaciones que optimizan propiedades funcionales clave.

En una segunda fase, partiendo de FsC-fp, se generó una librería de 147 mutantes simples mediante estrategias complementarias que combinan *machine learning*, modelos epistáticos basados en secuencias y análisis de enzimas de organismos hipertermófilos. Las mutantes se generaron por mutagénesis dirigida, se verificaron mediante secuenciación Nanopore y se produjeron en *Komagataella phaffii*. Tras su caracterización, se seleccionaron aquellas con mejores perfiles de actividad y estabilidad térmica para ensayos de despolimerización de PLA y PET.

Los resultados obtenidos están guiando ahora el diseño de mutantes combinadas, con el objetivo de generar cutinasas más robustas y eficientes, aptas para la aplicación industrial. En conjunto, este trabajo evidencia el potencial de integrar herramientas computacionales y la validación experimental en la ingeniería de enzimas aplicadas al reciclaje de poliésteres.

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Peste des Petits Ruminants Virus Disarms TRIM25 to Evade Antiviral Immunity

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Peste des petits ruminants (PPR) is a highly transmissible viral disease that primarily affects small ruminants, especially sheep and goats. The causative agent, the peste des petits ruminants virus (PPRV), has a remarkable ability to interfere with interferon (IFN) induction and signaling, thereby disabling the cellular mechanisms required to establish an antiviral state and weakening the adaptive immune response. Among the cellular factors involved in antiviral defense, TRIM25 stands out as a key E3 ligase essential for activating the RIG-I pathway, which triggers type I IFN production upon detection of viral RNA. TRIM25 also contributes to the formation of stress granules (SG), cytoplasmic structures that halt translation and promote activation of the PKR-mediated antiviral pathway. Previous transcriptomic analyses in ovine dendritic cells revealed a reduction in TRIM25 transcripts following PPRV infection, suggesting that this host factor might be directly targeted by the virus.

Our results confirmed that TRIM25 downregulation also occurs at the protein level in PPRV-infected sheep dendritic cells. The functional relevance of this observation was further supported by experiments in TRIM25-deficient THP-1 cells, where viral replication increased significantly, indicating that TRIM25 acts as an important restriction factor against PPRV.

To further elucidate the mechanisms underlying TRIM25 targeting by PPRV, we evaluated stress granule formation in infected cells. Infection with a virulent PPRV strain impaired SG induction, whereas cells infected with a vaccine strain retained the ability to form SG. To identify the viral components responsible for this effect, we performed immunoprecipitation assays with PPRV proteins and found that the non-structural protein C interacted with TRIM25. Interestingly, in PPRV-C–transfected cells, stress granule formation was impaired and TRIM25 appeared to co-localize with the viral protein.

These findings indicate that the non-structural protein C from PPRV may sequester TRIM25, thereby impairing its antiviral functions in both RIG-I activation and SG formation. The present work describes a new mechanism used by PPRV to prevent IFN induction and block the establishment of the antiviral state. Understanding how the virus circumvents the IFN system can help decipher the immunosuppressive mechanisms central to PPRV pathogenesis in small ruminants.

Contribución del segmento M del ARN de la cepa 40Fp8 del virus de la fiebre del Valle del Rift a la atenuación viral

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El virus de la fiebre del Valle del Rift (RVFV; género Phlebovirus) es un arbovirus emergente y zoonótico de importancia veterinaria y en salud pública. Su genoma está compuesto por tres segmentos de ARN monocatenario de sentido negativo: S (Small), M (Medium) y L (Large). Previamente, se generó una variante hiperatenuada de RVFV, denominada 40Fp8, mediante mutagénesis aleatoria en cultivo celular a partir de la cepa sudafricana virulenta 56/74. Aunque se han caracterizado mutaciones atenuantes en los segmentos L y S del 40Fp8, la contribución del segmento M sigue sin explorarse. El análisis de secuenciación de la cepa 40Fp8 reveló heterogeneidad genética en el segmento M, con secuencias consenso identificadas mediante secuenciación Sanger (consenso M1) y secuenciación de nueva generación (NGS; consenso M2), lo que sugiere una población de cuasiespecies. El objetivo de este estudio fue generar virus recombinantes de RVFV (rRVFV) que portaran las variantes consenso del segmento M de 40Fp8 y su posterior caracterización *in vitro e in vivo.* Los rRVFV se generaron mediante un sistema de genética inversa, combinando una de las variantes M1 o M2 con los segmentos S y L de la cepa 56/74. *In vitro*, los virus que portaban el segmento M de 40Fp8 presentaron placas significativamente más pequeñas y una cinética de replicación mayor que aquellos rRVFV con el segmento M de 56/74. La atenuación viral se evaluó en ratones 129Ev/Sv inoculados con ~10^2 PFU. Las hembras mostraron una supervivencia del 100 %, mientras que los machos presentaron mayor susceptibilidad, permitiendo discriminar los niveles de atenuación, observándose que rM1 presenta una mayor atenuación que rM2. Estos datos muestran que el segmento M de 40Fp8 contribuye significativamente a la atenuación viral. No obstante, la compleja dinámica de las cuasiespecies en el segmento M dificulta identificar residuos específicos responsables del fenotipo hiperatenuado completo de 40Fp8.

Detección y caracterización genética del virus de la fiebre hemorrágica de Crimea-Congo en garrapatas del oeste de España (2017, 2020-2024)

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El virus de la fiebre hemorrágica de Crimea-Congo (VFHCC) se detectó por primera vez en España en 2010, en garrapatas recogidas de ciervos en el suroeste de Cáceres. Desde entonces, esta región, considerada endémica, ha sido objeto de diversos estudios de vigilancia. Sin embargo, no se dispone de datos actualizados sobre la circulación viral en esta zona desde 2018. Con el objetivo de reevaluar la presencia y diversidad genética del VFHCC en Cáceres, se llevó a cabo un estudio de vigilancia retrospectivo en garrapatas recogidas en el centro y sur de la provincia durante varios periodos (2017 y 2020-2024). En total, se analizaron 3.183 garrapatas, agrupadas en 1.569 lotes, procedentes de ungulados silvestres, ganado, animales domésticos y vegetación. La detección viral se llevó a cabo mediante dos métodos de PCR, y los lotes positivos se caracterizaron mediante secuenciación por Sanger.

El VFHCC se detectó exclusivamente en *Hyalomma lusitanicum*, con una tasa de infección global del 1,54 % (IC 95 %: 1,14–2,03). La mayoría de las garrapatas positivas procedían de ungulados silvestres, mayoritariamente ciervos. El análisis genético reveló la circulación de dos genotipos de CCHFV, con predominio del genotipo III.

La detección de VFHCC a lo largo de varios años respalda su circulación sostenida en el suroeste de Cáceres. Estos hallazgos confirman el papel clave de *H. lusitanicum* en el mantenimiento del virus en la naturaleza y el de los ciervos como huéspedes amplificadores en el ecosistema. Asimismo, la detección de dos genotipos pone de manifiesto la diversidad genética de las cepas circulantes, así como la importancia de utilizar múltiples métodos moleculares. Estos resultados en conjunto enfatizan la necesidad de una vigilancia continuada en zonas endémicas para monitorizar la circulación viral y evaluar los riesgos para la sanidad animal y la salud pública.