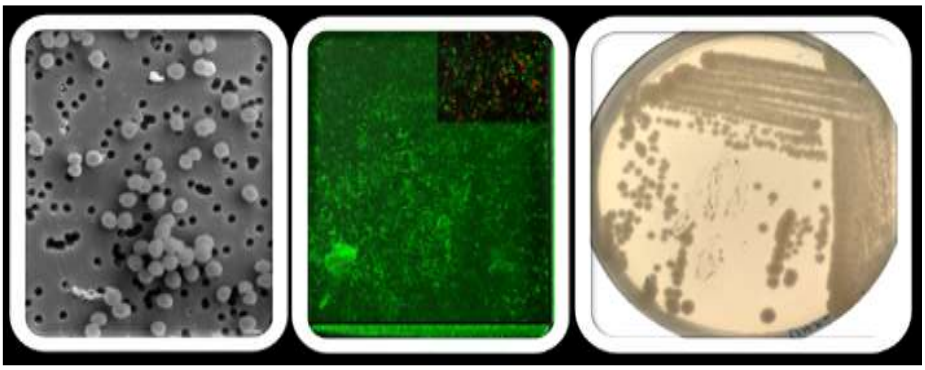
III Jornada de Microbiologia

Societat Catalana de BIOLOGIA

Institut d'Estudis Catalans

Organitzada per la Secció de Microbiologia de la

Societat Catalana de Biologia



INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

25 de Maig de 2022

III Jornada de Microbiologia

Organitzada per la Secció de Microbiologia de la Societat Catalana de Biologia

PROGRAMA

Comitè Organitzador

Eduard Torrents Serra
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Dimecres, 25 de Maig de 2022

09:00 – 09.15 Recollida de documentació

Sessió MATÍ

09:15 - 09:30 Inaguració de la **III Jornada de Microbiologia - 2022.**

Moderador: *Mireia López Siles (UdG)*

09:30 – 09:45 *Pathogenic bacteria in the human-chimpanzee-livestock interface in western Uganda*
Andrea Dias-Alves. Departament de Medicina i Cirurgia Animals. (UAB).

09:45 – 10:00 *Sunken riches: Ascomycete diversity in marine sediments of the Tarragona´s coast.*
Daniel Guerra-Mateo. Facultat de Medicina i Ciències de la Salut. (URV).

10.00 – 10:15 *Modification in grazer and viral pressure and decrease in resource competition leads to steep increase in the culturability of abundant marine bacteria.*
Xavier Rey-Velasco. Institut de Ciències del Mar (CSIC).

10:15 – 10:30 *Phage-based biocontrol of nitrification in agricultural soil*
Laura Sala-Comorera. Departament de Genètica, Microbiologia i Estadística. (UB).

10:30 – 10:45 *Potential new Aeromonas species isolated from the natural area "El Clot de la Mare de Déu".*
Roberto M.Guerra. Facultat de Medicina I Ciències de la Salut, IISPV. (URV).

10.45 – 11:00 *Exophiala caementiphila: a new species from darkened surface of a building wall In Els Pallaresos (Tarragona province, Spain).*
Sastoque, Angie. Facultat de Medicina i Ciències de la Salut. (URV).

11:00–11:45 Sessió de pòsters – Coffee break

Moderador: *Llorens Fernández (UdG)*

11:45 – 12:00 *Exploring the recent evolution of yeast pathogens using the CandidaMine database*
Miquel Àngel Schikora-Tamarit. Barcelona Supercomputing Centre (BSC)

12:00 – 12:15 *Novel finding on the activation of bulk autophagy through iron and glucose depletion in Saccharomyces cerevisiae.*
Sandra Montella-Manuel. Institut de Recerca Biomèdica (IRB-Lleida).

12:15 – 12:30 *In vitro activity and in vivo efficacy of flavone F46 alone or in combination with azoles against Candida auris.*
Youssef Ahmiane. Facultat de Medicina i Ciències de la Salut, IISPV. (URV).

12:30 – 12:45 *Diversity and phylogeny of onygenalean fungi in freshwater sediments from the Iberian Peninsula.*
Daniel Torres. Facultat de Medicina i Ciències de la Salut. (URV).

12:45 – 13:00 *Brown macroalgae (Phaeophyceae) extracts with antimicrobial activity as a natural strategy to improve food safety*
Susana Rubiño. Food Safety and Functionality Programme. (IRTA-Monells).

Moderador: *Jordi Mas (UAB)*

13:00 – 13:15 *Epidemiology of zoonotic Campylobacter from gulls from southern Spain and its public health relevance*.
Mª Pilar González-Navarro. Unitat mixta d'Investigació IRTA-UAB.

13:15 – 13:30 *High pressure processing as a control measure in pre-packed cooked ham. Is its efficacy affected by packaging?*
Cristina Serra-Castelló. Food Safety and Functionality Programme. (IRTA-Monells).

13.30 – 13.45 *Chicken livers at slaughter as a potential source of zoonotic Campylobacter*
Alicia Manzanares. Unitat mixta d'Investigació IRTA-UAB.

13:45–14:45 DINAR - Sessió de pòsters

Sessió TARDA

Moderador: *Marc Valls (UB / GRAC)*

14:45 – 15.00 *A novel acyl-homoserine-lactone acylase in Stenotrophomonas maltophilia with bifunctional activity.*
Marc Bravo. Institut de Biotecnologia i Biomedicina. (UAB-IBB).

15.00 – 15.15 *Enterobacter cloacae ATCC 13047 present two cheA-cheW clusters each one playing different roles in chemotaxis and pathogenicity.*
Frutos-Grilo, E. Departament de Genètica i Microbiologia. (UAB).

15:15 – 15:30 *Pseudomonas aeruginosa non-phosphorylated AlgR induces ribonucleotide reductase expression under oxidative stress conditions.*
Alba Rubio-Canalejas. Institute for Bioengineering of Catalonia (IBEC) I (UB).

15:30 – 15:45 *Ralstonia solanacearum dynamic gene expression throughout its life cycle*
Jordi Corral. Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB).

15:45 – 16:00 *Chromosome-level assemblies from diverse clades reveal limited structural and gene content variation in the genome of Candida glabrata.*
Marina Marcet-Houben. Barcelona Supercomputing Centre (BSC-CNS) and (IRB Barcelona).

16:00 – 16:15 *Isolation of plant-associated bacteria with potential to promote plant growth and stress tolerance.*
Kostadin E. Atanasov. Faculty of Pharmacy and Food Sciences. (UB).

16:15 – 16:45 Sessió de pòsters - Pausa

Moderador: *Anna Arís (IRTA)*

16:45 – 17:00 *A versatile and dynamic approach against antimicrobial resistant bacteria: the HDP-based multidomain proteins.*
Adrià López-Cano. Department of Ruminant Production, (IRTA-Caldes de Montbui).

17:00 – 17:15 *Study of the stabilization of protein-only nanoparticles containing antimicrobial peptides with liposomes and micelles.*
J. Atienza-Garriga. Institut de Biotecnologia i de Biomedicina. (UAB).

17:15 – 17:30 *Membrane vesicles from the probiotic Escherichia coli Nissle 1917 increase TFF3 expression by modulating TLR2 and miR-7- 5p.*
Olivo-Martínez Y. Facultat de Farmàcia i Ciències de l'Alimentació (UB) i Institut de Biomedicina (UB).

17.30 – 17:45 *Colonization of extended-spectrum beta-lactamase-producing Enterobacteriaceae in children: prevalence, colonization dynamics, persistence and impact on the intestinal microbiota.*
Mireia López-Siles. Departament de Biologia. UG.

17:45 – 18.00 Cloenda.
Eduard Torrents. Secció de Microbiologia, Societat Catalana Biologia, IEC

PÒSTERS

1. *Neutralization of ionic interactions by dextran-based single-chain nanoparticles improves tobramycin diffusion into a mature biofilm.* Núria Blanco-Cabra, Julie Movellan, Marco Marradi, Raquel Gracia, Cristian Salvador, Damien Dupin, Iraida Loinaz, **Eduard Torrents**. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

2. *Both human and soybean ferritins highly improve the accumulation of bioavailable iron and contribute to extend the chronological life in budding yeast.* Nuria, Pujol-Carrión, Alma, Gomez Alfonso, Sergi, Puig and Maria Angeles de la Torre-Ruiz. Cell Signalling in Yeast Unit, Department of Basic Medical Sciences, Institut de Recerca Biomèdica de Lleida (IRBLleida), University of Lleida, Lleida, Spain.

3. *Galleria mellonella as an alternative animal model for evaluating nanomaterials interactions and toxicology.* **Joana Admella**, Laura Moya-Andérico, José A. Del Río, Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

4. *Deciphering bacterial taxa to discriminate between subtypes of inflammatory bowel disease with colonic location.* **Mireia López-Siles**, Paula Torrent, David Busquets, Miriam Sabat-Mir, L. Jesús García Gil, Margarita Martínez-Medina. Grup de Microbiologia de la Malaltia intestinal, Departament de Biologia, Universitat de Girona, Girona, Spain.

5. *Study of the OmpA role in virulence of the AIEC (Adherent-Invasive Escherichia coli) pathotype.* Lara Ruiz Auladell, Carla Camprubí-Font, Toni Duran Pastor, Llorenç Fernández-Col, Margarita Martinez-Medina. Grup de Microbiologia de la Malaltia Intestinal, Universitat de Girona, Girona, Spain.

6. *Identification of new genes putatively implicated in the AIEC phenotype.* **Queralt Bonet-Rossinyol**, Carla Camprubí-Font, Mireia Lopez-Siles, Margarita Martinez-Medina. Microbiology of Intestinal Disease, Biology department, Universitat de Girona, Girona, Spain.

7. *A new BiofilmChip device as a personalized solution for testing biofilm antibiotic resistance.* Núria Blanco-Cabra, Maria José López-Martínez, **Betsy Verónica Arévalo-Jaimes**, María Teresa Martin-Gómez, Josep Samitier and Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

8. *Ultrastructural and compositional analyses of internal lipid inclusions in mycobacteria grown under different culture conditions.* **Víctor Campo-Pérez**, Sandra Guallar-Garrido, Marina Luquin, Alejandro Sánchez-Chardi and Esther Julián. Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain.

9. *Pseudomonas aeruginosa and Burkholderia cenocepacia multispecies biofilms: an in vitro model for airway infections.* **Julia Alcacer**, Nuria Blanco-Cabra, Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

10. *Host Defense Peptides-based molecules as new antimicrobial treatment for the multifactorial affection of Bovine Respiratory Disease.* **Ricardo Baltà-Foix**; Cristina Saubi; Anna Arís; Elena Garcia-Fruitós.Ruminant production group. Institut de Recerca i Tecnologia Agroalimentàries (IRTA).

11. *Functionalized nanoparticles for biofilm treatment***.** **Laia Rocher**, Joana Admella, Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

12. *Genome analysis of Candida orthopsilosis marine isolates unveils the missing parental lineage and suggests an environmental origin of hybrids with pathogenic potential.* **Valentina del Olmo**, Verónica Mixão, Ester Saus, Ewa Księżopolska, Juan Carlos Núñez and Toni Gabaldón. Barcelona Supercomputing Center (BSC) and Institute for Research in Biomedicine (IRB), Barcelona, Spain.

13. *Study and analysis of class Ia ribonucleotide reductase from Pseudomonas aeruginosa.* **Ángela Martínez-Mateos**, Alba Rubio-Canalejas, Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

14. *Recombinant host defense peptides modulate the cytokine secretion profile of epithelial cells.* **Cristina Saubi**, Ricardo Baltà-Foix, Elena Garcia-Fruitós and Anna Arís. Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Caldes de Montbui, Spain

15. *Do changes on formulation of dry-fermented sausages could be a concern for pathogens growth? The use of user-friendly tools to assess the impact of food safety.* **Anna Austrich-Comas,** Arícia Possas, Sara Bover-Cid, Anna Jofré. IRTA, Food Safety and Functionality Program, Finca Camps i Armet, Monells, Spain.

16. *First draft genome of Aphanoascus keratinophilus (Chrysosporium keratinophilum) obtained by hybrid assembly.* **Alan Omar Granados-Casas**, Angie Paola Sastoque Martínez, Ana Fernández-Bravo, Alberto Miguel Stchigel, José Francisco Cano-Lira*.* Unidad de Microbiología, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili, Reus, Spain.

17. *Impact of formulation and ripening process on the microbiota dynamics of nutritionally improved fuets.* **Núria Ferrer-Bustins**, Belén Martín, Sara Bover-Cid, Anna Jofré. Food Safety and Functionality Programme, Institute of Agrifood Research and Technology (IRTA), Monells, Spain.

18. *Meta-analysis of the microbial inactivation under non-thermal high pressure processing of fruit and vegetable juices and purees.* **Berta Torrents-Masoliver**, Cristina Serra-Castelló, Anna Jofré, Albert Ribas- Agustí, KahYen Claire Yeak, Heidy M.W. den Besten, Sara Bover-Cid. IRTA, Food Safety and Functionality Program, Monells, Spain.

19. *Role of mycobacteria-polarized macrophages in bladder cancer treatment.* Marc Bach-Griera, Manuela Costa, Margarida Saraiva, **Esther Julián***.* Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

20. *Assessment of the presence of potentially zoonotic bacterial pathogens in fecal samples of Pipistrellus kuhlii in connection to four differently degraded Mediterranean ecosystems.*

Beatriz Bellido-Martín, Lourdes Lobato-Bailón, Manuel García-Ulloa, Andres Santos Ñanculef, Jaime Martínez-Urtaza, Lourdes Migura-García, Johan Espunyes, Maria P. Ribas, Andrea Dias, Ignasi Marco, Oscar Cabezón. Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Catalonia, Spain.

21. *The long non-coding RNA landscape of Candida yeast pathogens.* **Hrant Hovhannisyan**, Toni Gabaldón. Barcelona Supercomputing Center (BSC) and Institute for Research in Biomedicine (IRB), Barcelona, Spain.

22. *Longitudinal study of rectal microbiota in calves with or without diarrhea episodes before weaning.* **Pau Obregon-Gutierrez**, Jaume Bague-Companys1, Alex Bach, Virginia Aragon, Florencia Correa-Fiz. Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Catalonia. Spain.

23. *pETS-IBECGLOW: A new generation of bacteria promoter-probe and transposon-delivery plasmids.* **Domingo Marchan del Pino**, Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

24. *Easily applicable modifications to electroporation conditions improve the transformation efficiency rates for rough morphotypes of fast-growing mycobacteria.* **Víctor Campo-Pérez**, Maria del Mar Cendra, Esther Julián and Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

25. *Remodelling the bladder immune microenvironment by mycobacterial species with changes in their cell envelope composition.* Sandra Guallar-Garrido, Jordi Senserrich, Elisabet Gomez-Mora, Víctor Urrea, Bonaventura Clotet, Thierry Soldati, Cecilia Cabrera, **Esther Julián**. Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

26. *3D spatial organization and improved antibiotic treatment of a Pseudomonas aeruginosa – Staphylococcus aureus wound biofilm by nanoparticle enzyme delivery.* **Alba Rubio-Canalejas**, Aida Baelo, Sara Herbera, Núria Blanco-Cabra, Marija Vukomanovic and Eduard Torrents. Institute for Bioengineering of Catalonia (IBEC) and Universidad de Barcelona. Barcelona, Spain.

27. *Methodologies for the identification of keystone species from microbiome datasets and co-occurrence networks.* ***Maravall-Lopez, Javier****; Casanellas, Marta; Casacuberta, Carles; Gabaldón, Toni.* Barcelona Supercomputing Center (BSC) and Institute for Research in Biomedicine (IRB), Barcelona, Spain.

28. *Monitoring microbial community dynamics of a rotary drum bioreactor with immobilized Trametes versicolor developed for agricultural wastewater treatment.* **Martí Pla-Ferriol**, Eduardo Beltrán-Flores, Paqui Blánquez, Montserrat Sarrà, Nuria Gaju, Maira Martínez-Alonso. Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona.

Societat Catalana de BIOLOGIA

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COMUNICACIONS ORALS

Presentació ORAL 1

Pathogenic bacteria in the human-chimpanzee-livestock interface in western Uganda

Andrea Dias-Alves[1], Manuel García-Ulloa[2], Andres Santos Ñanculef[2], Carol Asiimwe[3,4], Daniel Sempebwa[4], John Walter Akankwasa[4], Edrine Kayaga[5], Anna Malavé[1], Sebastian Napp[6,7], Lola Pailler[6,7], Jaime Martínez-Urtaza[2], Lourdes Migura-Garcia[6,7], Ignasi Marco[1], Oscar Cabezón[1,6]

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[2] Departament de Genètica i Microbiologia, UAB, 08193 Bellaterra, Spain
[3] The Jane Goodall Institute - Uganda, P.O Box 462, Entebbe, Uganda
[4] Budongo Conservation Field Station, P.O. Box 362, Masindi, Uganda
[5] Central Diagnostic Laboratory, COVAB, Makerere University, P.O. Box 7062 Kampala, Uganda
[6] Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalonia, Spain
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Zoonotic diseases are caused by pathogens naturally transmitted between animals and humans and present a major threat to global public health, as well as to the health and well-being of livestock and wildlife. The impact of these diseases in developing countries is extremely high due to population growth, lack of infrastructure and capacity to tackle disease outbreaks, habitat degradation, and high human-animal interaction. Chimpanzees are increasingly exposed to human pathogens, which can be detrimental to the conservation of this endangered species. In this study, the presence of potential pathogenic bacteria in the gut of 39 chimpanzees, 20 humans and 20 goats from Budongo Forest, a tropical rain forest located in Western Uganda, and its neighboring areas is assessed through 16S ribosomal RNA (rRNA) sequencing. In total, 8 bacterial genera were identified: *Campylobacter*, *Clostridium*, *Salmonella* and *Shigella* spp. were detected in all species, *Helicobacter* and *Klebsiella* spp. were not detected in goats and chimpanzees, respectively, whereas *Listeria* spp. was only present in humans, and *Pseudomonas* spp. in chimpanzees. *Clostridium perfringens*, *Salmonella enterica*, *Shigella (S.) boydii*, *S. dysenteriae* and *S. flexneri* were detected in the three species analyzed. Our results suggest that habitat overlap can influence the transmission of pathogens crossing the interspecies barrier. Future research should focus in this area since big data analysis throughout sequencing could help disease prevention and the implementation of risk mitigation strategies in areas with high human-chimpanzee-livestock interaction.

Presentació ORAL 2

Sunken riches: Ascomycete diversity in marine sediments of the Tarragona´s coast

Daniel Guerra-Mateo[1], Josepa Gené Díaz[1], Vladimir Baulin[2], José F. Cano Lira[1].

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[2] Universitat Rovira i Virgili, Facultat de Química, Física i Cristal·lografia de Materials, Tarragona, Spain.

Little is known about the fungal composition of Mediterranean marine sediments. Culture independent studies suggest that this substrate comprises a great fungal diversity, where ascomycetes are predominant over other filamentous fungal groups. However, only a small fraction of this diversity is known and available in culture. In this work, we present a survey of marine sediments collected at four points in front of the Tarragona´s coast (Catalonia). Each point was defined at a different depth in the water column based on two sediment types: sand (6 m and 13 m) and silt (20 m and 27 m). Samples were processed independently through both direct plating and a flocculation pre-treatment, cultured on DRBC, MEA3% and PDA with actidione and incubated at 15ºC and 25ºC. Fungal identifications were based on micromorphology and rDNA sequence analyses performed mainly with the barcodes ITS and LSU. We obtained 188 isolates that represent around 36 genera, where *Aspergillus* (30%), *Penicillium* (12%), and *Queenslandipenidiella* (6%) were the most common. Silt sediments provided the greatest amount of both isolates and diversity (75% and 89%, respectively), but both sediment types provided isolates of taxonomical interest. We have delineated, through a polyphasic approach, four novel taxa that belong to the genera: *Amphichorda* (*Cordycipitaceae*), *Exophiala* (*Herpotrichiellaceae*), *Malbranchea* (*Myxotrichaceae*) and *Queenslandipenidiella* (*Teratosphaeriaceae*); and we recovered *Byssoonygena ceratinophila* (*Onygenaceae*), a rare species previously known only from two specimens collected in garden soil. The combination of direct plating and flocculation with different culture media helped to maximise the detectable diversity through culture. These results suggest that Mediterranean marine sediments could represent a reservoir of interesting ascomycetes, which diversity can be assessed through culture-dependent techniques.

Presentació ORAL 3

Modification in grazer and viral pressure and decrease in resource competition leads to steep increase in the culturability of abundant marine bacteria

Xavier Rey-Velasco[1], Adrià Auladell[1], Ona Deulofeu[1], Isabel Sanz-Sáez[1], Josep M. Gasol1, Olga Sánchez[2]

[1] Department of Marine Biology and Oceanography, Institut de Ciències del Mar (CSIC), 08003 Barcelona, Spain)
[2] Departament de Genètica i Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Isolation of relevant microorganisms is a useful tool to gather knowledge about their ecology, physiology and genomic properties, in addition to characterize novel taxa. To date, marine bacterial isolation efforts have been made directly from environmental samples, mainly taxa from the 'rare biosphere'. In this study, we have performed an extensive isolation effort on samples from manipulation experiments that were carried out during the four astronomical seasons in the coastal NW Mediterranean to evaluate the impact of grazing, viral mortality, resource competition and light on bacterioplankton growth. Isolates were cultured in two media with different properties and their full 16S rDNA was sequenced intending to (a) obtain an isolate collection, (b) assess how environmental conditions affect isolation success and (c) isolate bacteria exhibiting a blooming behavior (i.e. fast growth) after the treatments. A total of 1643 isolates were obtained, mainly affiliated to classes *Gammaproteobacteria* (44%), *Alphaproteobacteria* (26%) and *Bacteroidia* (16%). The most commonly isolated genera were *Alteromonas* and *Limimaricola*. Compared to the controls, isolates pertaining to class *Gammaproteobacteria* were more abundant in all experiments, while *Bacteroidia* were enriched in the predator-reduced treatments. 83 isolates had a similarity below 97% to public databases and their novelty will be further tested. Culturability increased significantly in nutrient-enriched and virus-reduced treatments, reaching 1.4% of all cells in the fall virus-reduced treatment. Comparison of isolate sequences with high-throughput 16S rDNA sequencing amplicon reads showed that the percentage of reads corresponding to isolates dramatically increased up to 70% and 47% in the summer virus-reduced and nutrient-enriched treatments respectively. Finally, we isolated 11 taxa that became dominant (bloomers) in the different experiments belonging to genera *Alteromonas*, *Vibrio*, *Limimaricola* and *Polaribacter*. In conclusion, our study indicates that grazer and viral pressure as well as nutrient availability are key factors affecting isolation success in marine environments.

Presentació ORAL 4

Phage-based biocontrol of nitrification in agricultural soil

Laura Sala-Comorera[1], Pablo Quirós[1,2], Clara Gómez-Gómez[1,3], Lorena Rodríguez-Rubio[1], Tula Yance-Chávez[2], Sergio Atares[2], Sandra Garcia-Gutierrez[3,4], Sonia Garcia[3,4], Antonio Vallejo[3,4], Ignasi Salaet[2], Maite Muniesa[1]

[1] Departament de Genètica, Microbiologia i Estadística. Universitat de Barcelona, Barcelona, Spain
[2] Departamento de I+D+i de Fertinagro Biotech S.L., Teruel, Spain
[3] Departamento de Química y Tecnología de Alimentos, ETSI Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain
[4] Centro de Estudios e Investigación para la Gestión de Riesgos Agrarios y Medioambientales, Universidad Politécnica de Madrid, Madrid, Spain

Nitrogen fertilization is a necessary but low efficient process to increase crops yields. The loss of useful nitrogen forms by the quick activity of nitrifying bacteria forces to use high amounts of nitrogen fertilizers. This excess of nitrogen fertilizers leads to the emergence of harmful nitrogen forms such as NO_3 and N_2O that seriously contaminate the environment. Chemical inhibitors of nitrifying bacteria are, so far, the more effective strategy to improve the fertilization efficiency, but the effects of such compounds on the environment and on human health are yet not well stablished. This study proposes a new strategy based on the use of bacteriophages that infect and eliminate nitrifying bacteria. This study describes the isolation of virulent bacteriophages infecting several species of *Nitrosomonas*. The phage selected, called ΦNF-1, inhibits the bacterial growth and causes a significant inhibition of the nitrification process that results in a reduction of the ammonium depletion in the phage-treated cultures tested in vitro and in soil samples. The application of phages as biocontrol agents might be a useful strategy for the inhibition of nitrification while overcoming the restrictions related to the application of chemical compounds.

Presentació ORAL 5

Potential new *Aeromonas* species isolated from the natural area "El Clot de la Mare de Déu"

Roberto M.Guerra[1], Francisco Damián Maleno[1], Isabel Pujol[1,2], Maria José Figueras[1], Ana Fernández-Bravo[1]

[1]Unitat de Microbiologia, Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, Reus, Tarragona, Spain
[2]Laboratori Microbiologia, Hospital Universitari Sant Joan de Reus, Entitat de Dret Públic (EDP) Salut Sant Joan de Reus-Baix Camp, Reus, Tarragona, Spain

Aeromonas are autochthonous microorganisms of the aquatic environment considered emerging pathogens for humans, causing a wide spectrum of diseases. Previous taxonomy studies based on single housekeeping genes such as *rpoD* or *gyrB* have provided coherent phylogenies of *Aeromonas*. However, the sequencing of only one gene may not confer enough resolution to show, without doubt, the phylogenetic positions of some closely related species within this bacterial genus. An *Aeromonas* strain (A7) isolated from an environmental water source from the natural area "El Clot de la Mare de Déu" in Burriana, Spain, could not be assigned to any known species of this genus based on *rpoD* gene sequences. The Multilocus Phylogenetic Analysis (MLPA) of the concatenated partial sequences of seven genes (*rpoD*, *gyrB*, *recA*, *dnaJ, gyrA*, *dnaX,* and *atpD*; 3949 bp), using the Neighbor-joining method suggested that this strain has a significant distance (94.97% similarity) from its closest related species, *Aeromonas popoffii* type strain CECT 5176, to be considered a different species. This strain could be differentiated from the closest *Aeromonas* species based on several phenotypic characteristics, particularly arginine dehydrolase activity and the fermentation of carbohydrates rhamnose, salicin, and sucrose. Collectively, through this polyphasic approach comparing the phenotypic characterization and phylogenetic analysis with seven housekeeping genes, we conclude that this strain (A7) could represent a novel species within the genus *Aeromonas*. However, further studies will be performed such as genome sequencing to support this new species, using bioinformatics tools (*in silico* DNA-DNA hybridization and Average nucleotide identity).

Presentació ORAL 6

Exophiala caementiphila: a new species from darkened surface of a building wall in Els Pallaresos (Tarragona province, Spain).

Sastoque, Angie[1], Alberto Stchigel[1], José Cano-Lira[1]

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In order to identify the responsible fungi for the darkening of certain buildings in Els Pallaresos village (Tarragona province, Spain), a representative number of samples were taken from the affected surfaces using moistened cotton swabs. Once in the laboratory, the samples were inoculated by streaking on different culture media (DRBC, PCA, PDA and TWA) by duplicate, and incubated at two different temperatures (15 and 37 ºC) in order to isolate the greatest diversity of fungal taxa. To obtain pure cultures, material from the colonies was taken using sterile needles and transferred to sterile culture media, which were incubated in the same conditions than previously. The fungal strains were phenotypically characterized and a preliminary molecular identification was carried out by means of amplification and sequencing of the ITS-LSU region of the rRNA genes. The strain FMR 18977 caught our attention, when its vegetative and reproductive structures were examined under bright field microscopy. The colonies were black, circular, glistening at first, but becoming opaque, dry, rugose and cerebriform with the age, and the mycelium almost absent, submerged, with branched, cylindrical or torulose hyphae. The conidiogenous cells were annellidic, mono- to polyblastic, integrated to the hyphae or discrete, then laterally disposed, and the conidia enteroblastic, becoming conidiogenous cells over time. Yeast-like cells were very abundant, developing secondary conidia to form long chains. In order to identify this fungus, a maximum-likelihood phylogenetic tree using ITS nucleotide sequences was built. The results demonstrated that our strain FMR 18977 represents a new species of the genus *Exophiala*, being phylogenetically close related to *E. crusticola*, exhibiting the defining characters of the genus, but also distinctive of the other species already described. Consequently, we propose the new species *Exophiala caementiphila*, from Latin *caementum*-, cement, and -*phila*, friendship, because the origin of the fungus.

Presentació ORAL 7

Exploring the recent evolution of yeast pathogens using the CandidaMine database

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Advances in medicine (such as chemotherapy or transplants) have extended the life expectancy of patients at the cost of impairing their immune system. This has generated an increasing population of patients highly susceptible to infections. Among them, fungal infections caused by *Candida* species have become a major life-threatening issue, with insufficient diagnostic and therapeutic options. Recent studies have used population genomics in clinical *Candida* isolates to understand their recent evolution, which may also clarify the emergence of phenotypes like drug resistance or virulence. However, there are open questions that hinder our understanding about such evolutionary processes. We tried to clarify some of these questions by generating variant calling data for ~2,000 isolates from seven major *Candida* pathogens, available at the CandidaMine database. We focused on understanding 1) the role of structural variants (SVs), 2) the genomic determinants of antifungal drug resistance, 3) the role of recombination, 4) the population structure and 5) the similarities among these processes in different *Candida* species. Importantly, our collection represents a higher sample size as compared to previous studies, suggesting that we have unprecedented power. Interestingly, we found that SVs drive a significant amount of genetic variation, suggesting that their role should not be overlooked. In addition, we found novel clades and recombination events in several species. Finally, our inter-specific comparison analysis revealed important differences between species, which may be relevant to develop species-tailored diagnostics or therapies. In summary, our work improves the understanding of the recent evolution of the major *Candida* pathogens.

Presentació ORAL 8

Novel finding on the activation of bulk autophagy through iron and glucose depletion in *Saccharomyces cerevisiae*

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Autophagy is a tightly regulated mechanism involving several signaling pathways. Iron is an essential metal for the majority of cellular types. It is required for many metabolic processes such as respiration, proteins, lipids or ribosome metabolism, DNA biosynthesis and repair and others. Iron depletion causes health problems in humans such as cardiovascular diseases. Autophagy activation in response to iron starvation is a positive mechanism to recirculate iron. We have demonstrated that iron limitation provokes one primary response inducing bulk autophagy mediated by TORC1. The signal of iron deprivation requires TORC2/Ypk1, the yeast orthologues of mammalian mTORC2/Sgk1, activity and the inactivation of Tor1 leading to Atg13 dephosphorylation, thus triggering the autophagy process. Iron depletion contributes to the extension of the chronological life, in a manner totally dependent on autophagy activation.

Carbon sources have a major impact on *Saccharomyces cerevisiae* metabolism and also affect longevity. Glucose limitation induces slowing growth, contributing to switch to respiratory metabolism, the hallmark of the diauxic shift, which along with other metabolic processes prepares cells for the stationary phase and the process of chronological ageing. Mtl1 is a cell wall receptor belonging to the CWI pathway. Mtl1 function is related to glucose and oxidative stress signaling. In this context, we show that bulk autophagy is highly induced during the transition to diauxic shift in a manner totally dependent on glucose starvation. The Mtl1 receptor is essential to sense glucose concentration and transmit the signal to Ras2, the orthologous to the mammalian Ras2, and Sch9 to phosphorylate atg1 and to activate the macroautophagy machinery. All the above suggest a pivotal signaling role for Mtl1 in maintaining correct cell homeostasis function is periods of glucose scarcity in budding yeast being a good target as antifungal therapy.

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Presentació ORAL 9

In vitro activity and in vivo efficacy of flavone F46 alone or in combination with azoles against *Candida auris.*

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Candida auris is an emerging, nosocomial multidrug-resistant threat with high treatment failure and mortality rate that has spread quickly worldwide. Accordingly, there is an urgent need for developing new therapies and flavones could be an alternative by their demonstrated antimicrobial activity. Therefore, we evaluated the *in vitro* activity of flavone F46 alone or in combination with azoles against *C. auris* and its efficacy in a murine model of *C. auris* disseminated infection.

Antifungal activity of F46 alone or in combination with fluconazole (FLC), voriconazole (VRC) and posaconazole (PSC) against 4 *C. auris* strains was evaluated following CLSI guidelines. Antibiofilm activity of F46 was also determined by the crystal violet quantification method. The *in vivo* efficacy of anidulafungin (AFG: 10 mg/kg QD) and F46 (20 mg/kg QD) alone or combined with PSC (20 mg/kg BID) was assessed in a murine model of systemic infection using 2 strains. Animal survival and reduction of fungal load in kidneys, liver and lungs was analysed on days 8 and 24 post-infection.

In vitro drug interaction (FICI) showed synergistic or additive activity of F46 with PSC or VRC, while indifferent effect was observed with FLC. Non-antibiofilm activity of F46 was detected. Experimental treatment shown efficacy of AFG, PSC and PSC+F46 to decrease mortality of immunocompromised mice (100% of survival) compared to control animals (25%). Besides, AFG, and PSC+F46 treatment reduced significantly burdens of *C. auris* in studied organs from 8 days post-infection. The combination resulted in the highest CFU reduction and showed a good ability to sterilise lung and liver. F46 monotherapy did not show efficacy in reduction of fungal load.

The combination of flavone F46 with azoles showed a synergistic to additive effect *in vitro* against *C. auris* as corroborated by *in vivo* studies, although the combination needs to be optimised to increase its efficacy.

Presentació ORAL 10

Diversity and phylogeny of onygenalean fungi in freshwater sediments from the Iberian Peninsula

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The order *Onygenales* is a well-known group of filamentous ascomycetes since it includes many species of medical interest able to cause animal and human infections in healthy and immunosuppressed individuals. They commonly inhabit soils and have especial predilection for keratinic substrates due to their ability to decompose and assimilate this nutrient compound. Some previous studies exploring fungal diversity from freshwater environments revealed that sediments could be a reservoir of this interesting group of ascomycetes. Therefore, we present here the results on our ongoing survey of cultivable filamentous fungi focused on the study of onygenalean species diversity from freshwater sediments collected in rivers and streams of natural areas in the Iberian Peninsula. We have collected 107 samples of sediments mainly from Aragon, Balearic Islands, Catalonia, Madrid and Valencian Communities. To achieve a greater fungal diversity, we used different culture media, including potato dextrose (PDA) supplemented with cycloheximide and dichloran rose bengale chloramphenicol (DRBC). A total of 56 isolates of onygenalean fungi were mainly recovered from the former culture medium. They were identified morphologically and by DNA sequence analyses of the ITS and LSU nrDNA regions as species of the genera *Amauroascus, Aphanoascus, Arachnotheca, Atrhroderma, Arthropsis, Chrysosporium, Emmonsiellopsis, Gymnoascus, Gymnoascoideus, Leucothecium, Malbranchea, Myriodontium* and *Polytolypa*. However, several isolates could not be assigned to any known species and are recognized as putative novel taxa for the *Onygenales*. A multi-locus analysis, combining sequences of the ITS, LSU, fragments of the β-tubulin (*tub*2) and fragments of the RNA polymerase II subunit (*rpb*2), as well as a comparative morphological study have allowed us to elucidate their taxonomy and to propose at least five new species for the science. These results confirm that the freshwater sediments from our country are rich on onygenalean fungi, including species of taxonomic interest.

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Presentació ORAL 11

Brown macroalgae (Phaeophyceae) extracts with antimicrobial activity as a natural strategy to improve food safety

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Growing consumer demands for high-quality products with longer shelf-life, coupled with minimally processed products and a less use of synthetic food additives, promote that the development of alternative solutions for food safety continue playing a key role in food industry. Consequently, efforts are currently under way for the search of new sources of natural antimicrobials. In this sense, brown macroalgae represent an unexploited resource of bioactive compounds since characteristic structures from this macroalgae group like phlorotannins, fucoidans or fucoxanthin have been widely described as potential antimicrobial compounds.

The aim of this study was to assess the antimicrobial potential of extracts obtained from brown macroalgae collected in North coasts of Spain, as well as the evaluation of their potential as food preservatives.

Macroalgae extracts were performed from 20 brown macroalgae species using a mid-polarity extraction medium. Their antimicrobial potential was first tested by disk diffusion assay and Minimum Inhibitory (MIC) and Minimum Bactericidal Concentrations (MBC) were evaluated for the active extracts. Although a high variability was observed due to environmental effects, extracts from *Bifurcaria bifurcata*, *Dictyota dichotoma* and *Ericaria selaginoides* showed activity against six Gram-positive target strains including *Bacillus cereus*, *Bacillus subtilis*, *Geobacillus stearothermophilus, Listeria monocytogenes, Staphylococcus aureus* and *Staphylococcus haemolyticus,* selected because of their interest as food-borne pathogens and spoilage bacteria. Antimicrobial activity of the purified apolar fraction from *E. selaginoides* extracts was further evaluated as ingredient for extending the safe shelf-life of fresh cheese against *L. monocytogenes*. The results obtained through by a challenge test showed that the effect over this pathogen was dose- dependent and its growth was completely inhibited at the highest concentration tested.

This study proves that brown macroalgae constitute an alternative source of antimicrobial compounds of natural origin with application as effective food preservatives to improve the safety and extent shelf-life of ready-to-eat products.

Keywords: Brown macroalgae, bioactive compounds, antimicrobial activity, food safety.

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Presentació ORAL 12

Epidemiology of zoonotic *Campylobacter* from gulls from southern Spain and its public health relevance

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Some gull species, particularly those with scavenging habits, can act as reservoirs of infectious agents, playing an important role in their dissemination and maintenance in the environment. This includes zoonotic *Campylobacter* spp., the leading cause of food-borne disease in developed countries worldwide. To gain insight into *Campylobacter* epidemiology, we sampled 69 healthy individuals from two gull species, year-round in Málaga (south Spain): Lesser black-backed gulls (*Larus fuscus*, n= 48) and Yellow-legged gulls (*Larus michahellis,* n= 21). Also, to understand the relationship between health status and *Campylobacter* prevalence, we sampled 36 and 33 Lesser black-backed gulls and Yellow-legged gulls, respectively, found with paretic syndrome. We assessed the *Campylobacter* prevalence and the genetic diversity of the isolates. Of the overall 138 birds sampled, *Campylobacter* prevalence was 22%, with Yellow-legged gulls showing the highest prevalence (13%). We detected *C. jejuni* and *C. lari*, and coinfections with both species with a prevalence of 65%, 29% and 6%, respectively. Notably, in both gull species *Campylobacter* prevalence was lower in healthy individuals than in those suffering from paretic syndrome: 8% vs 25% in Lesser black-backed gulls, 14% vs 45% in Yellow-legged gulls, suggesting sick gulls seem to be more susceptible to infection. The genetic diversity of *Campylobacter* was studied by FlaA-RFLP and compared with those from gulls sampled in other Spanish localities. We found a high genetic diversity among isolates from Málaga, but also the same strains (same RFLP profile) from this locality in other Spanish localities, suggesting both gull species have a high potential to spread *Campylobacter* strains over long distances across the Iberian Peninsula. This is consistent with the migratory behaviour of these species, with the former being migratory while Yellow legged gull is partially migratory. Overall results highlight gull species may pose a public health risk, especially in a human populated area.

Presentació ORAL 13

High pressure processing as a control measure in pre-packed cooked ham. Is its efficacy affected by packaging?

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From the microbiology point of view, the perishability of pre-packed cooked ham is attributed to the growth of spoilage and pathogenic microorganisms that limit the sensory and the safe shelf-life. High pressure processing (HPP) can be used by cooked ham manufacturers as a non-thermal preservation technology to inactivate microorganisms; however, its efficacy needs to be assessed considering the intrinsic and extrinsic characteristics of the product.

The aim of the study was to assess the impact of packaging (under vacuum, VP and modified atmosphere, MAP) on the HPP-inactivation kinetics of the most relevant pathogen (*Listeria monocytogenes*) and spoilage (lactic acid bacteria) microorganisms in cooked hams with different formulations. For this, *L. monocytogenes* CTC1034 and *Lactilactobacillus sakei* CTC746 (slime producer) were inoculated on slices of cooked hams, packaged in vacuum and MAP (N2:CO2, 80:20) and pressurized (400MPa/0-15min) 1h (VP, MAP) or 24h (MAP-exposed) after packaging. Chromogenic agar and MRS were used to enumerate *L. monocytogenes* and *L. sakei*, respectively. HPP-inactivation kinetics were characterized with the Weibull model.

The results showed that the HPP-inactivation of both microorganisms was enhanced in MAP compared to VP cooked hams. In MAP-exposed samples, the enhanced inactivation of *L. monocytogenes* observed in MAP samples was reversed indicating that the longer exposure to MAP allowed the pathogen to adapt to HPP. On the contrary, an enhanced *L. sakei* inactivation by HPP was observed in MAP-exposed products, reducing up to 65% compared to that of products pressurized 1h after MAP application. Therefore, the type of packaging and the time period between packaging and HPP raise as relevant factors affecting the HPP-inactivation in cooked ham, though with different impact for the pathogen (protecting) and the spoilage (enhancing) bacteria.

Presentació ORAL 14

Chicken livers at slaughter as a potential source of zoonotic Campylobacter

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Campylobacter is the most common cause of human gastroenteritis worldwide. Poultry and poultry products are the main source of infection. Outbreaks of campylobacteriosis attributed to undercooked chicken livers have been reported worldwide. In Spain there is a lack of information on the occurrence of *Campylobacter* in chicken livers. Thus, we aimed to determine the prevalence and levels of *Campylobacter* in these edible tissues by sampling 56 flocks from two slaughterhouses in Catalonia, northeastern Spain. We collected three carcasses per flock randomly during the evisceration of the animals, analyzing in total 168 liver and ceca samples. We compared the prevalence of *Campylobacter* from cecal contents, liver surface and inner liver tissue. Overall, *Campylobacter* prevalence was 54,8% in cecal samples, whilst in the liver surface and the internal tissue was 71,4% and 35,1%, respectively. Out of the 92 *Campylobacter*-positive cecal samples, 45,7% were *C. coli*-positive, 48,9% were *C. jejuni*-positive and in the remaining 5,4% of these samples, coinfections with both bacteria were identified. Among the 120 *Campylobacter*-positive samples of the external surface of the liver, 40,0% were positive to *C. coli*, 45,8% were positive to *C. jejuni* and in the remaining 14,2%, we found coinfections with both bacteria. Finally, out of the 59 *Campylobacter*-positive samples of internal tissue liver, a 37,3% were *C. coli*-positive, a 52,5% were *C. jejuni*-positive, and in 10,2% of samples both bacteria were detected. The data highlights chicken livers as a potential source of human campylobacteriosis, not only due to the *Campylobacter* prevalence but particularly because of the bacterial load, which was $>10^3$ CFU/liver in 40,1% of the samples of surface liver and $>10^3$ CFU/g in the 6,6% of the internal tissue samples. Further research is needed to determine the risk of campylobacteriosis due to consumption of chicken livers.

Presentació ORAL 15

A novel acyl-homoserine-lactone acylase in *Stenotrophomonas maltophilia* with bifunctional activity

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Acyl-homoserine-lactone acylases (AHL acylases) are enzymes responsible for disrupting the quorum sensing communication between bacteria by hydrolysis of AHL signaling molecules. A novel enzyme capable of degrading AHLs in the multidrug-resistant opportunistic bacterium *Stenotrophomonas maltophilia* has been identified by homology to AHL acylases previously described in other bacteria. Heterologous expression in *Escherichia coli* of this new enzyme has demonstrated its AHLs degrading activity *in vivo*, while a mutant strain of *S. maltophilia* K279a deficient for this enzyme showed a loss of this activity. During the production of the protein in *E. coli*, the enzyme was shown to consist of two subunits as described in other known acylases. Until now, the production of AHLs by *S. maltophilia* has been questioned due to the lack of homologues of known AHL synthase family genes, but the fact that detectable levels of AHLs have been identified in supernatants of *S. maltophilia* deficient for this AHL acylase could indicate the existence of a new family of synthases. In addition to having identified a new quorum quenching mechanism in these bacteria, the protein heterologously expressed in *E. coli* has been found to be capable of degrading β-lactams, indicating that it is a bifunctional enzyme that could confer antibiotic resistance in *S. maltophilia.*

Presentació ORAL 16

Enterobacter cloacae ATCC 13047 present two *cheA-cheW* clusters each one playing different roles in chemotaxis and pathogenicity

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Chemotaxis not only enables bacteria to move according to chemical gradients but also plays a role in their virulence. Despite this, very little is known about the chemotactic ability of *Enterobacter* spp., a nosocomial and multidrug-resistant pathogen that has been pointed out by WHO as one of the critical pathogens to what new and alternative therapies must be obtained to allow the treatment of its infections.

Our studies have revealed the presence of two active copies of *cheA* and *cheW* genes, both encoding proteins belonging to the chemotactic signaling complex. The chemotaxis and swimming essays confirmed that one of the gene clusters is responsible for the ability to orient the bacteria towards favorable nutrient gradients. In fact, the phylogenetic analysis pointed out that the *cheA2-cheW2* cluster is more prevalent in *Enterobacter* than *cheA1-cheW1*, which appeared during the Enterobacteriaceae speciation, and it is only conserved in some bacterial species.
In contrast, the overexpression of the *cheA1-cheW1* promotes changes in surface motility of *Enterobacter*. Furthermore, the expression levels of these genes change when *Enterobacter* is adhered to or is invading T24 bladder cells, indicating its involvement in bacterial virulence.
Thus, our results indicate that while one of the *cheA-cheW* copies seems to be involved in the classical chemotaxis pathway, the other one plays a role in other mechanisms associated with virulence, such as motility and eukaryotic cell adherence and invasion. Our data enlightens the knowledge of *Enterobacter* pathogenicity as well as open the door for the pursuit of new therapeutic strategies capable of blocking infection by interfering with chemotactic signaling pathways of this emerging pathogen.

Presentació ORAL 17

Pseudomonas aeruginosa non-phosphorylated AlgR induces ribonucleotide reductase expression under oxidative stress conditions

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Ribonucleotide reductases (RNR) are key enzymes that catalyze the synthesis of deoxyribonucleotides, the monomers needed for DNA replication and repair. RNR are classified into three classes (I, II, and III) depending on their overall structure and metal cofactor. Organisms whose genome encodes for several RNR classes are remarkably adaptable to different environments. *Pseudomonas aeruginosa* is an opportunistic pathogen harboring all three classes of RNR, increasing its metabolic versatility. It is known that during an infection, *P. aeruginosa* can grow to form a biofilm to be protected from the host immune defenses, such as the production of reactive oxygen species by macrophages. One of the essential transcription factors needed to regulate biofilm growth and other metabolic pathways is AlgR. AlgR is part of a two-component system where FimS is a kinase that catalyzes its phosphorylation in response to external signals.
Additionally, AlgR is part of the regulatory network of the cell RNR regulation. In this study, we delved into the regulation of the RNR through AlgR under oxidative stress conditions. We have determined that the non-phosphorylated form of AlgR is responsible for the class I and II RNR induction after H_2O_2 addition in planktonic culture and during flow biofilm growth. We observed a similar RNR induction pattern comparing the *P. aeruginosa* laboratory strain PAO1 with different *P. aeruginosa* clinical isolates. And finally, we shown that during a *Galleria mellonella* infection, where oxidative stress is highly produced, AlgR was crucial to induce class II RNR gene (*nrdJ*). Thereby, we showed that the non-phosphorylated form of AlgR, besides being crucial for infection chronicity, regulates the RNR network in response to oxidative stress during infection and biofilm formation.

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Presentació ORAL 18

Ralstonia solanacearum dynamic gene expression throughout its life cycle

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Ralstonia solanacearum is a phytopathogenic bacterium responsible of the bacterial wilt disease, which causes important crop yield and economic losses worldwide. Disease management remains limited by the faculty of the pathogen to survive for years in soil or water ponds before colonizing new plant hosts. Despite the main bacterial virulence determinants and metabolic pathways required for the life within the plant have been thoroughly studied, little is known about the life of *R. solanacearum* outside the host.

In the present work, we present RNA-sequencing data of *R. solanacearum* recovered from soil and water and compare the obtained data with previously published transcriptomes of the bacterium growing inside the plant. We show that *R. solanacearum* overexpresses in soil stress response and bacterial defense genes, whereas secretion systems and translation are strongly repressed. On the contrary, *R. solanacearum* surviving in water upregulates genes involved in defense, inorganic ion transport, flagellum and, surprisingly, the type 3 secretion system (T3SS). This is the first study describing *R. solanacearum* gene expression outside its plant hosts, which are key for its survival in the environment.

Presentació ORAL 19

Chromosome-level assemblies from diverse clades reveal limited structural and gene content variation in the genome of *Candida glabrata*

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Candida glabrata is an opportunistic yeast pathogen displaying a large genetic and phenotypic diversity and a highly plastic genome. However, the lack of chromosome-level genome assemblies representing this diversity limits our ability to accurately establish how chromosomal structure and gene content vary across strains. Here, we expanded publicly available assemblies by using long-read sequencing technologies in twelve diverse strains, obtaining a final set of twenty-one chromosome-level genomes spanning the known *C. glabrata* diversity. Using comparative approaches, we inferred variation in chromosome structure and determined the pan-genome, including an analysis of the adhesin gene repertoire. Our analysis uncovered four new adhesin orthogroups, and inferred an ancestor encoding a rich adhesion repertoire, which was subsequently shaped through a still ongoing process of gene loss, gene duplication, and gene conversion. *C. glabrata* has a largely stable pan-genome except for a highly variable subset of genes encoding cell-wall associated functions.

Presentació ORAL 20

Isolation of plant-associated bacteria with potential to promote plant growth and stress tolerance.

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Land plants are soil-dependent organisms with sessile lifestyle that interact with a plethora of multi-kingdom microorganisms. These interactions are crucial to adapt to the environment, grow and develop. Some soil-born bacteria can colonize the plant host and establish complex relationships that can be categorized as neutral, commensal, mutualistic, beneficial, or even pathogenic. Roots are the main gates for microbial colonization of plants. Root colonization niches can be divided in zone of root influence (rhizosphere), root surface/epithelium (rhizoplane), or the inner root-tissue. For instance, when an organism is found living and growing within the plant tissue, and does not produce disease symptoms, this microbe is defined as endophyte, a term deriving from the Greek words "*endon*" (within) and "*phyton*" (plant). Endophytic bacteria play crucial roles in promoting plant growth and stress tolerance by stimulating nutrient mobilization and hormonal responses, among other mechanisms. Beneficial plant microbiota is currently being used for the development of new plant biostimulants that promote growth and stress protection while reducing the need to use of chemical fertilizers in agriculture, which is an important source of environmental pollution. This strategy is part of the European Green Deal, aiming at the development of a more sustainable and eco-friendly agriculture while providing solutions to the adverse environmental conditions driven by climate change.

Presentació ORAL 21

A versatile and dynamic approach against antimicrobial resistant bacteria: the HDP-based multidomain proteins

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Antibiotic effectiveness is steadily compromised due to the emergence of antimicrobial-resistant (AMR) bacteria and the scientific community has pooled its efforts in the pursuit of novel alternatives. Among them, the host defense peptides (HDPs) that are short cationic peptides of the innate immunity, hold compelling features to address the issue, including a broad antimicrobial spectrum, along with rapid and multiple modes of action. However, their inherent lability, and high manufacturing cost associated with chemical synthesis hamper a broader implementation. In this regard, recombinant production opens up a promising alternative to achieve cost/effective compounds with improved characteristics. Going a step further, this recombinant technology enables the combination of multiple domains with interesting features in a single molecule with a plethora of opportunities. Thus, the aim of this study was the development of a novel platform to produce proteins with a broad-spectrum antimicrobial activity against AMR bacteria, combining single-domain HDPs (first generation proteins) to build up an enhanced and fully tailored second generation of multidomain proteins. The first approaches showed that two multidomain proteins (D5L37βD3 and D5L37D5L37) exhibited strong bactericidal activities against *Staphylococcus aureus* methicillin-resistant (MRSA), *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* methicillin-resistant, even in those bacteria forming biofilms. Thus, considering the outcomes observed, further studies will be performed to evaluate the role of each domain in the final construct as well as the significance of their relative position or repetitions in the multidomain protein.

Presentació ORAL 22

Study of the stabilization of protein-only nanoparticles containing antimicrobial peptides with liposomes and micelles

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Antimicrobial peptides (AMPs) are secreted factors involved in the function of the innate and acquired immune system, providing potent efficacy against bacteria, fungi, and viruses. AMPs work by interacting with negatively charged surfaces of infectious agents, thereby destabilizing biological structures due to their cationic and amphiphilic properties. Interaction of bacterial membrane with AMPs compromises cell integrity. The potential therapeutic value of AMPs and formulations containing AMPs has therefore been explored.

A small peptide like an AMP is difficult to be recombinantly produced at large scale. It has been demonstrated that peptides can be fused to a scaffold protein as an alternative to overcome this limitation. A modular recombinant protein derived from this process can be efficiently produced and purified.

Recombinant gene engineering can direct the synthesis of proteins that form protein nanoparticles (NPs) through the expression of novel genes. When the AMPs are forming part of NPs, they retain their biological activity and are envisioned as potential therapeutic formulations in infectious diseases.

However, in respiratory tract infections, the administration of medicines is hampered by the presence of the mucus barrier which is continuously moving and can potentially block the access of the active molecule to the target infectious organism. Therefore, novel formulations need to be developed to overcome these limitations.

In this study, AMPs fused to His-tagged eGFP were studied for their solubility pattern at different pHs and their loading capacity, followed by their stability in liposomes and micelles, with the goal of obtaining novel formulations that can protect protein nanoparticles from protease activity when administered intranasally.

Presentació ORAL 23

Membrane vesicles from the probiotic *Escherichia coli* Nissle 1917 increase TFF3 expression by modulating TLR2 and miR-7- 5p

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Trefoil factor 3 (TFF3) is a small peptide secreted by intestinal Globet cells that plays a key role in the maintenance and repair of intestinal mucosa. Reduced levels of TFF3 were detected in damaged tissue of IBD patients. TFF3 expression is upregulated through the TLR2 pathway and downregulated by miR-7-5p. Our group showed that administration of membrane vesicles (MVs) released by the probiotic *Escherichia coli* Nissle 1917 (EcN) increase TFF3 expression in murine models of experimental colitis.

This study aims at evaluating the molecular mechanisms involved in the regulation of TFF3 by EcN MVs. The LS174T cell line, which displays a Globet cell phenotype, is suitable to approach TFF3 expression analysis. LS174T cells were stimulated with MVs from EcN or the commensal EcoR12. Expression of TLR-2, miR-7-5p and TFF3 were evaluated by RT-qPCR, and the secreted TFF3 protein by ELISA. EcN MVs significantly increased TLR2 and TFF3 mRNA levels, and differentially downregulated miR7-5p. Consistently, TFF3 levels were higher in culture supernatants of LS174T cells stimulated with EcN MVs than those from control or cells treated with EcoR12 MVs. Next, the conditioned medium (GC- CM) from control and stimulated LT174T cells were used to analyse their effect on the strengthening of tight junctions (TJs) in Caco-2 cells. EcN-derived GC-CM positively modulated epithelial barrier integrity through upregulation of the TJ proteins ZO-1, occludin and claudin-1. Treatment with EcoR12-derived GC-CM did not cause any effect. Moreover, wound healing assays showed that EcN-derived GC-CM promoted repair of damaged intestinal epithelium.
In conclusion, this study proves that EcN MVs increase TFF3 expression through TLR2 activation and miR7-5p downregulation. Subsequently, secreted TFF3 positively modulate both expression of TJ proteins and wound healing of intestinal epithelial cells. By these mechanisms, EcN MVs help to maintain integrity of the intestinal mucosa.

Presentació ORAL 24

Colonization of extended-spectrum beta-lactamase-producing Enterobacteriaceae in children: prevalence, colonization dynamics, persistence and impact on the intestinal microbiota.

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Extended-spectrum ß-lactamase-producing Enterobacteriaceae (ESBL-E) colonize the intestinal tract, being able to cause infections or transfer resistance to other commensal microorganisms. Despite ESBL-E infections reported in children have increased in recent years, and approved antibiotic treatments for this age group are limited, few studies have explored ESBL-E distribution in this population.

Our aim was to determine the prevalence, duration of colonization, impact on the intestinal microbiota and the characteristics of ESBL-E strains colonizing non-hospitalized children.

Prevalence of ESBL-E colonization was determined in 1011 school children (0-13 years old). A subpopulation of 124 children was analyzed bimonthly to assess duration of colonization. Fecal samples were screened for ESBL-E carriage in selective medium. ESBL-E isolates were characterized by establishing PFGE profile, MLST, *bla* allele and antibiotic resistance profile. Gut bacterial community was analyzed in a subpopulation of 24 ESBL-E carriers *vs* a paired counterpart of non-ESBL-E carriers by amplification of the V3-V4 regions of the 16S rRNA gene, and sequencing using the Illumina MiSeq platform.

ESBL-E colonization rate was 2.6±1.0%, with duration from <2-6 months. All ESBL-E isolates were *Escherichia coli*. A high clonal diversity was observed among them (<60% PFGE profile similarity). The *bla*$_{\text{CTX-M}}$ alleles were the most frequent. ESBL-E isolates were resistant to trimethoprim/sulfamethaxazole (53%), gentamicin (14%), ciprofloxacin (31%), and levofloxacin (31%). No differences were detected in the richness, diversity or composition at the phylum level of the intestinal microbiota between ESBL-E carrier and non-carrier children.

The prevalence of ESBL-E in the studied cohort is lower than the colonization rate previously detected in Europe. ESBL-E colonization episodes lasted <2 months, and the existence of persistent colonization could be demonstrated for a maximum period of 6 months, without impacting the intestinal microbiota of non-hospitalized children. Co-resistance to other antibiotics of routine use in clinical practice is frequent in ESBL-E isolated from children.

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PÒSTERS

Pòster 1

Neutralization of ionic interactions by dextran-based single-chain nanoparticles improves tobramycin diffusion into a mature biofilm

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The extracellular matrix protects biofilm cells by reducing diffusion of antimicrobials. Tobramycin is an antibiotic used extensively to treat *P. aeruginosa* biofilms, but it is sequestered in the biofilm periphery by the extracellular negative charge matrix and loses its efficacy significantly. Dispersal of the biofilm extracellular matrix with enzymes such as DNase I is another promising therapy that enhances antibiotic diffusion into the biofilm. Here, we combine the charge neutralization of tobramycin provided by dextran-based single-chain polymer nanoparticles (SCPNs) together with DNase I to break the biofilm matrix. Our study demonstrates that the SCPNs improve the activity of tobramycin and DNase I by neutralizing the ionic interactions that keep this antibiotic in the biofilm periphery. Moreover, the detailed effects and interactions of nanoformulations with extracellular matrix components were revealed through time-lapse imaging of the *P. aeruginosa* biofilms by laser scanning confocal microscopy with specific labeling of the different biofilm components.

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Pòster 2

Both human and soybean ferritins highly improve the accumulation of bioavailable iron and contribute to extend the chronological life in budding yeast.

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Ferritin proteins have an enormous capacity to store iron in cells. In search for the best conditions to accumulate and store bioavailable iron, we made use of a double mutant null for the monothiol glutaredoxins GRX3 and GRX4. The strain grx3grx4 accumulates high iron concentrations in the cytoplasm, making the metal easily available for ferritin chelation. Here we perform a comparative study between human (L and H) and soybean ferritins (H1 and H2) function in the eukaryotic system Saccharomyces cerevisiae. We demonstrate that the four human and soybean ferritin chains are successfully expressed in our model system. Upon co-expression of either both human or soybean ferritin chains, respiratory conditions along with iron supplementation, led us to obtain the maximum yields of iron stored in yeast described to date. Human and soybean ferritin chains are functional and present equivalent properties as promoters of cell survival in iron overload conditions. The best system revealed that the four human and soybean ferritins possess a novel function as antiaging proteins in conditions of iron excess. In this respect, both ferritin chains with oxidoreductase capacity (human-H and soybean-H2) bear the highest capacity to extend life suggesting the possibility of an evolutionary conservation.

Pòster 3

Galleria mellonella as an alternative animal model for evaluating nanomaterials interactions and toxicology

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The use of nanomaterials in consumer products is currently on the rise, so it is important to have reliable methods to predict any associated toxicity effects. Traditional *in vitro* assays fail to mimic true physiological responses of living organisms against nanomaterials whereas murine *in vivo* models are time consuming, costly and ethically controversial. Therefore, alternatives must be considered. Here we present the insect *Galleria mellonella, an* infection animal model with a promising potential for the toxicological evaluation of different molecules and materials. It presents an array of attractive advantages as it has a convenient size for manipulation, it is inexpensive to purchase and breed, does not require much space or special infrastructure, it has a low biohazard risk and it is more ethically accepted. Moreover, a wide range of methodologies can be applied for visualizing and studying nanoparticles in this organism and several indicators can be obtained from the larvae to define the degree of acute toxicity effects that are caused by the different kinds of particles and materials. We believe this model can be used as a bridge between *in vitro* and *in vivo* murine assays to obtain better predictions of nanomaterials toxicity.

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Pòster 4

Deciphering bacterial taxa to discriminate between subtypes of inflammatory bowel disease with colonic location

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The dysbiosis of Crohn's disease (CD) and ulcerative colitis (UC) patients differ. Although the location of the disease is relevant to clinical practice, few studies have explored the changes in the microbial community among the subtypes of inflammatory bowel disease (IBD) that affect the colon (E1, E2, E3, C-CD, and IC-CD).

Our aim was to analyze the richness, diversity, and composition of the microbiota associated with the colonic mucosa of IBD patients with colonic disease and identify bacterial markers that allow their discrimination.

Colonic biopsies were obtained from 21 UC (5 E1, 6 E2, and 10 E3) and 22 CD (12 C-CD and 10 IC-CD) patients. The mucosa-associated microbial community was analyzed by sequencing the V4 region of the 16S rRNA gene by Illumina MiSeq. Richness and diversity indices and the relative abundance of taxa at different taxonomic levels (phylum-genus) were determined by disease subtype. The usefulness of the significant taxa as indicators to distinguish amongst different disease subtypes was established by the area under the ROC curve (AUC).

Richness (Sobs=55-288) and diversity (InvSimp=1.9-38.9) of the bacterial community is similar among all the disease subtypes. Six indicators with differences in relative abundances between subtypes have been identified (relative Ab=0.16-20.91%). The abundance of indicators 36 and 75 was significantly lower in patients with C-CD than those with UC of any location ($p \leq 0.040$, $AUC \geq 0.726$). Besides, indicators 2, 11, and 69 showed significant differences between E3 and C-CD ($p \leq 0.015$, $AUC \geq 0.829$). Indicators 2 and 11 also showed differences between UC locations ($p \leq 0.017$, $AUC \geq 0.861$), while the relative abundance of indicators 51 and 19 is higher in C-CD compared to IC-CD ($p \leq 0.007$, $AUC \geq 0.846$).

A differential microbiological signature between colonic IBD subtypes has been identified. Quantification of these taxa would allow refining the use of microbiomarkers to support IBD diagnosis and assess the risk of disease extension.

Pòster 5

Study of the OmpA role in virulence of the AIEC (Adherent-Invasive *Escherichia coli*) pathotype

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Adherent-invasive *Escherichia coli* (AIEC) pathotype has been associated with Crohn's disease, an inflammatory bowel disease of unknown etiology. AIEC adhere and invade intestinal epithelial cells (IECs); and survive and replicate within macrophages without producing host cell death. Variations in the sequence and/or the expression of outer membrane proteins (OMPs) may modulate bacterial virulence. OmpA has been involved in the interaction of AIEC strain LF82 with IECs, and five mutations have been shown to play a role in a better interaction with the receptor (Gp96) (Rolhion et al 2010). The aim of our work is to investigate whether OmpA is an important virulence factor in AIEC strains carrying different OmpA variants. We have sequenced 14 AIEC and 30 non-AIEC strains and identified 13 variants. No particular variants were found specific of AIEC. Nonetheless, we performed OmpA isogenic mutants in AIEC strains using the λ-Red recombinase method and determined their adhesion and invasion abilities on Intestine-407 cells. We achieved isogenic mutants from five AIEC strains, two carrying the variant 7 and three the variant 14. All isogenic mutants showed a decrease in the adhesion and invasion abilities on IECs cultures. This result supports the hypothesis that OmpA play a role in AIEC virulence. Further experiments of transcomplementation of non- AIEC K-12 strain-OmpA sequence variant present in AIEC strains with the AIEC LF82 strain-OmpA sequence variant will demonstrate whether these mutations are definitely important for a better adhesion/invasion.

Pòster 6

Identification of new genes putatively implicated in the AIEC phenotype

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Adherent invasive *Escherichia coli* (AIEC) have been related to Crohn's disease. Since no molecular tools are available for AIEC identification, phenotypic assays based on its capacity of adhesion and invasion to cellular cultures are used. We hypothesize that differential gene expression may drive to the AIEC phenotype. To test this hypothesis we investigated differentially expressed genes (DEGs) between AIEC and non-AIEC strains and evaluated their suitability as molecular markers.

Comparative transcriptomics was performed between two AIEC/non-AIEC strain pairs during Intestine-407 cells infection. Each pair displayed identical pulsotypes and similar genomes. Supernatant fractions of the infected cell cultures (SN) and eukaryotic cells containing adhered and/or intracellular bacteria (A/I) were considered separately. DEGs obtained were quantified by RT-qPCR in the same samples to confirm the results, and in a strain collection of 13 AIEC and 23 non-AIEC. Binary logistic regression was used to identify DEGs whose quantification could be used as AIEC biomarker.

Comparative transcriptomics revealed 67 DEGs between AIEC and non-AIEC in the strain pairs, 51 of which (82.26%) were corroborated by RT-qPCR. In the whole strain collection, 29 DEGs were found between the two phenotypes ($p < 0.042$), and 42 DEGs between SN and A/I fractions ($p < 0.049$). Notably, six DEGs were implicated in the synthesis of two virulence determinants, and five in a metabolic pathway that could be involved in acid resistance. Moreover, binary logistic regression revealed three DEGs able to predict the AIEC phenotype with an accuracy of ≥ 85%.

No previous comparative transcriptomic studies have been performed using AIEC-infected cell cultures. We have identified DEGs that could be involved in AIEC pathogenicity and used as biomarkers. These results open the door to further research in AIEC biomarkers and in new therapeutic targets against AIEC colonisation.

Pòster 7

A new BiofilmChip device as a personalized solution for testing biofilm antibiotic resistance

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Currently, three major circumstances threaten the management of infections: increasing antimicrobial resistance, expansion of chronic biofilm-associated infections, and lack of an appropriate approach to treat them. To date, the development of accelerated drug susceptibility testing of biofilms and of new antibiofouling systems has not been achieved despite the availability of different methodologies. There is a need for easy-to-use methods of testing the antibiotic susceptibility of bacteria that form biofilms and for screening new possible antibiofilm strategies.

Here, we present a new easy method for testing antibiofilm susceptibility using a microfluidic device (BiofilmChip) that measures biofilm biomass by electrical impedance spectroscopy. BiofilmChip enables the growth of different bacterial species from clinically isolated strains and directly from sputum samples obtained from cystic fibrosis patients. Our results demonstrate that BiofilmChip is a useful tool for antimicrobial resistance testing in biofilms.

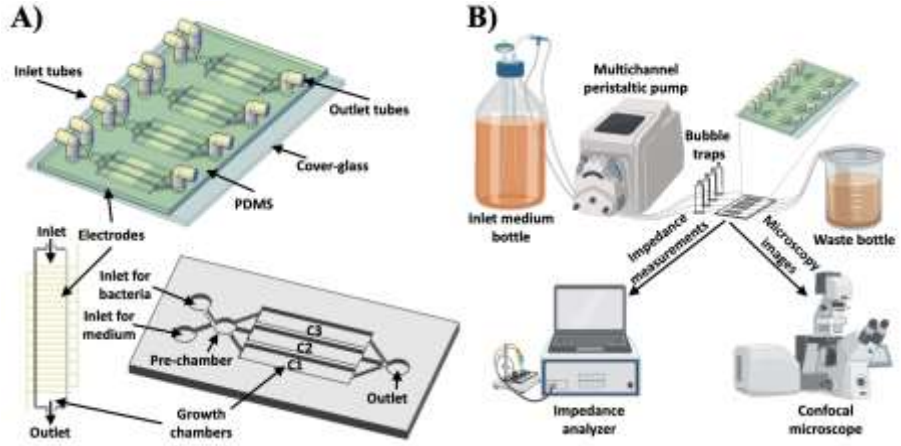

FIGURE: A) Biofilm chip design and B) experimental setup.

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Pòster 8

Ultrastructural and compositional analyses of internal lipid inclusions in mycobacteria grown under different culture conditions

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The formation of intracellular lipid inclusions (ILIs) in mycobacteria is relevant and it is especially studied and related to infection of pathogenic mycobacteria, especially *Mycobacterium tuberculosis*. However, little is known about ultrastructure, production, composition, and modulation of those ILIs, especially in environmental species such as *Mycolicibacterium brumae*. Here, we combined lipidic profiles of *M. brumae* grown at different culture media and development states with qualitative and quantitative approaches of both ultrastructural imaging (TEM, FESEM) and composition (BSE, EDX) to understand ILIs formation and dynamics. Our results show that culture media composition has a clear impact in ILIs dynamics affecting its formation, amount, and morphometry. At compositional level, low glycerol concentrations correlate with lower molecular weight and small size ILIs, contrary to those obtained in glycerol enriched media. High plasticity of lipidic profiles in *M. brumae* indicates its great versatility to adapt at different conditions reacting with metabolic changes that ends in differential lipidic patterns.

Pòster 9

Pseudomonas aeruginosa and *Burkholderia cenocepacia* multispecies biofilms: an in vitro model for airway infections

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Multispecies biofilms are communities composed of different microorganisms embedded in an auto-synthesized polymeric matrix. This matrix allows potential inter-species interactions that play an important role in chronic infections. *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* are two multidrug- resistant and biofilm-forming opportunistic pathogens often found in the lungs of cystic fibrosis patients. There is a gap in existing biofilm-related literature between *in vitro* single-species biofilm studies and extensive metagenomic studies. In this context, a stable and balanced *in vitro* model to study *P. aeruginosa* and *B. cenocepacia* multispecies biofilms has been built following three steps. First, both species were studied in planktonic cocultures, where *P. aeruginosa* appeared to dominate, and *B. cenocepacia* lost its viability after 24 h of incubation. Then, the effects of the initial inoculum concentration, the growth media conditions, and the incubation time were evaluated in *in vitro* static biofilms. Finally, a microfluidics-based dynamic biofilm formation system was used to test the optimal conditions for static multispecies biofilms. A stable and balanced multispecies biofilm was obtained: 60 % of the biomass corresponded to *P. aeruginosa* and 40 % corresponded to *B. cenocepacia*, and both species' viability was maintained after 72 h of incubation. Such a model will potentially shed light on the effects of one population over the other in chronic infections and to test novel antimicrobial therapies to combat *P. aeruginosa* and *B. cenocepacia* polymicrobial infections.

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Pòster 10

Host Defense Peptides-based molecules as new antimicrobial treatment for the multifactorial affection of Bovine Respiratory Disease

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The prevalence of infectious diseases has exponentially increased in the last decades. These diseases are caused by microorganisms, being bacteria one of the main pathogenic agents. The use and misuse of antibiotics has caused the appearance of resistant microorganisms, which are not sensible to the existing drugs. Within the context of cattle industry, the Bovine Respiratory Disease (BRD) represents a clear example of a multifactorial infectious disease caused by several types of bacteria and affecting a significative number of animals without any effective treatment. Aiming to develop an alternative therapeutic approach against complex bacterial infections, such as those causing BRD, we have developed an approach based on the use of Host Defense Peptides (HDPs) as antimicrobial agents. These small molecules are part of the innate immunity and perform a huge variety of functions in the host, including antibacterial activity, antiviral action, and modulation of the immune response. Thus, the main objective of the study is the development of a new type of drugs based on HDPs with a broad-spectrum antibacterial activity. A catalogue of HDPs including beta-defensins and cathelicidins have been recombinantly produced using *Lactococcus lactis* as expression system. Then, their antibacterial activity has been tested against relevant pathogens such as Methicillin Resistant *Staphylococcus aureus* (MRSA) or bacteria involved in BRD such as *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*. A significant reduction of the bacterial growth has been observed in most of the cases. The beta-defensin 1 and the cathelicidin BMAP27 have presented the best performance against MRSA. For *P. multocida* all proteins have been highly effective, while for *M. haemolytica* and *H. somni* beta-defensins have shown higher activity than cathelicidins. These results prove the potential of recombinant HDPs as robust antimicrobials with a broad-spectrum activity to treat infectious caused by pathogenic bacteria, including those resistant to antibiotics.

Pòster 11

Functionalized nanoparticles for biofilm treatment

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The use of nanoparticles (NPs) in the biomedical field is currently increasing, just like bacterial antibiotic resistance. The main form of resistance in chronic infections is the formation of biofilms. In this work, poly-lactic acid (PLA) NPs coated with poly-L-lysine (PLL) and functionalized with enzyme A were designed, and its capacity to disrupt static biofilms was tested with both *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms.

Pòster 12

Genome analysis of *Candida orthopsilosis* marine isolates unveils the missing parental lineage and suggests an environmental origin of hybrids with pathogenic potential

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Hybridisation, i.e. the crossing of two lineages to form a hybrid with an admixed genome, is a common event in yeasts often leading to genomic variability and adaptation. *Candida orthopsilosis* is a human-associated opportunistic pathogen belonging to the Candida parapsilosis species complex. Most clinical isolates from this species are hybrids resulting from at least four independent crosses between two parental lineages of which only one has been identified. The rare presence or total absence of parentals amongst clinical isolates has been hypothesised to be a consequence of a reduced pathogenicity with respect to their hybrids. Here, we analyse the genomes of the first sequenced environmental *C. orthopsilosis* strains, which were isolated from warm marine ecosystems. We found a majority of hybrid strains among environmental isolates, and we determined they are phylogenetically closely related to hybrid clinical isolates. Furthermore we identified the long-sought missing parental lineage, thus providing a complete overview of the genomic evolution of this species. Our results suggest a marine origin of *C. orthopsilosis* and pave the way to identify the pre-existing environmental adaptations that rendered hybrids more prone to colonise and infect the mammalian host.

Pòster 13

Study and analysis of class Ia ribonucleotide reductase from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a versatile opportunistic pathogen that easily adapts to changing environments. For that reason, and along with increasing antimicrobial resistance, there is an urgent need to find new therapies to combat these infections. **Ribonucleotide reductases (RNRs)**, a family of metalloenzymes involved in dNTP synthesis, is a key enzyme for life and represents a suitable target to fight this microorganism. **Class Ia RNR** of *P. aeruginosa* is encoded by the *nrdAB* operon, regulated allosterically and transcriptionally by NrdR, AlgR, and DnaA. However, nowadays, there are still some gaps in its regulation. It has been described a **long 5'UTR** (untranslated region) on *nrdA* mRNA, and it is reasonable to think that this 5'UTR region plays a key role in *nrdA* regulation. Bioinformatic analysis suggested that 5'UTR could be a **cobalamin riboswitch** and/or an **small RNA** (sRNA). Experimental assays do not confirm this first hypothesis. However, the second hypothesis was verified by RT-PCR. It is known that sRNAs have plenty of functionalities, the most noteworthy are mRNA stability and half-life. Via a shut-off transcriptional assay was proved that 5'UTR decreased *nrdA* **mRNA half-life**. Nevertheless, several experiments should be performed to exclude or confirm one or both hypotheses, apart from additional tests and further bioinformatics analysis.

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Pòster 14

Recombinant host defense peptides modulate the cytokine secretion profile of epithelial cells

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Host defense peptides (HDPs) are short and cationic peptides from the immune system proposed to be a potential alternative to antibiotics, originally because of their direct microbicidal properties. However, recent research has evidenced a primary role in modulating immune responses under physiological conditions. Multiple immunomodulation activities have been reported, including the direct stimulation and differentiation of immune cells, along with the regulation of cytokine secretion by various cell types. The current study investigated the effects of recombinant mammalian HDPs on the cytokine production of epithelial cells. In this regard, Caco2 cells were challenged for 24 h with four β-defensins (BD-1, 2, and 3, and the lingual antimicrobial peptide -LAP-); the defensin alpha 5 (HD-5); the surfactant protein D (SP-D); and the anionic peptide SAAP. A multiplex immunoassay was employed to quantify the cytokine profile of the cell culture supernatants. Results indicated a prevailing increase of interleukin-8 (IL-8) under the treatment of HDPs, with the exceptions of BD-2 and HD-5. Notably, BD-2 enhanced the production of interferon (IFN-γ) and interleukin 4 (IL-4), whereas HD-5 stimulated interleukin 6 (IL-6). This effect was magnified when combining HD-5 with an enzymatic antimicrobial peptide (sPLA2) and a bacterial binding domain (gelsolin) in the form of a multidomain protein (JAMF2). Not only greater levels of IL-6 were detected, but JAMF2 also significantly boosted the release of IL-8, IL-4, and tumor necrosis factor-alpha (TNF-α). These results indicate that HDPs alter the cytokine secretion of epithelial cells in a peptide-dependent manner. Hence, distinct cytokine profiles could determine whether the response is pro- or anti-inflammatory and at the innate or adaptive level. Furthermore, the stronger effect of HD-5 when included in a multidomain protein suggests that the use and combination of various HDPs could be a potential strategy to design new molecules with a wide range of immunomodulatory effects.

Pòster 15

Do changes on formulation of dry-fermented sausages could be a concern for pathogens growth? The use of user-friendly tools to assess the impact of food safety

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Clean labels or nutritionally improved formulations of dry-fermented sausages (FS) are increasing on demand. Their microbial safety can be assessed through the application of predictive models integrated in user-friendly tools. Stakeholders need to introduce the intrinsic and extrinsic factors as inputs to simulate the microbial response in terms of growth or inactivation.

The aim of this work was to evaluate the behaviour of foodborne pathogens associated with modifications of FS formulation: fat reduction, sodium chloride (NaCl) reduction/replacement and nitrite reduction/elimination, using user-friendly tools available at http://www.dmripredict.dk.

According to the "Yersinia enterocolitica" tool, a fat reduction from 20% to 10% only 0.3 Log_{10} units of difference on the inactivation was predicted. "ConFerm" tool allowed to comparatively quantify the impact of the reduction of NaCl concentration from 3.5% to 1% on the inactivation of *Salmonella* (3.7 Log_{10} less), *Escherichia coli* (5.1 Log_{10} less) and *Listeria monocytogenes* (1.4 Log_{10} less), being the later the most resistant. However, from the technological perspective, NaCl is usually replaced with equimolecular concentration of other salts and minimal impact in the pathogen inactivation was predicted when assessing 2% NaCl substitution for 2.5% KCl (0.2 Log_{10} difference). The effect of reducing nitrite from 150 ppm to 50 ppm or total removal was stronger in *L. monocytogenes* than in enteric pathogens, decreasing the predicted inactivation as nitrite-amount was reduced. In general, the acid FS process (pH=4.8) enhanced the inactivation extent (>0.3 Log_{10} units) for all pathogens compared to low-acid FS process (pH=5.4). Based on "Staphtox predictor" tool, at fermentation temperature of 22 ºC, 100 and 150 ppm of nitrite were needed to prevent *S. aureus* growth in acid and low-acid FS, respectively.

These predictive tools can be applied to assess and manage the microbiological hazards of innovative FS from the production process to retail to assure compliance with current regulation.

Pòster 16

First draft genome of *Aphanoascus keratinophilus* (*Chrysosporium keratinophilum*) obtained by hybrid assembly

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The genus *Aphanoascus* belongs to the order Onygenales and the family Onygenaceae. Some of these species are of great interest due to their important role as opportunistic pathogens for animals and humans and their production of multiple metabolites with biotechnological interest. In the last years, whole-genome sequencing has increased due to rapidly dropping sequencing costs, the ability to produce large volumes of data, and higher throughput. However, a few sequence genomes of this family are available, for this reason, the aim of the present study was to obtain the first draft of the complete genome of *Aphanoascus keratinophilus* (anam. *Chrysosporium keratinophilum*) using a hybrid approach. The genomic DNA of the type strain of *C. keratinophilum* (CBS 104.62) was extracted using the modified DNeasy ® Plant Mini Kit protocol (Qiagen). Later, the genome was sequenced using Illumina NovaSeq6000 paired-end sequencing technology combined with the Pacbio Sequel I platform. Subsequently, the data were assembled using the SPAdes and MaSuRCA tools. Our results showed that the best assembly was performed with MaSuRCA, resulting in a genome assembly with a size of 27.9 Mb, composed of 29 contigs with an N50 = 2,118,842 bp and a GC content of 49.2 %. In addition, the BUSCO analysis reported an integrity score of 95.9%, using lineage data from Onygenales_odb10. This study is the first report of the genome sequence of *A. keratinophilus* and this genomic data will allow future research in functional genomics and comparative genomics

Pòster 17

Impact of formulation and ripening process on the microbiota dynamics of nutritionally improved *fuets*

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Consumer preferences are changing towards nutritionally improved (i.e. salt and nitrite-reduced) processed meat products, posing a challenge to the food industry in terms of technological and food safety aspects. The aim of the present study was to evaluate the effect of sodium-reduced and nitrite-free formulations and low-temperature process on the dynamics of the bacterial communities of *fuet* by metataxonomics. Eight baches of *fuet* were manufactured with standard, sodium- reduced, nitrite-free (with/without a liver-based ingredient as substitute of technological function) formulations and submitted to ripening at mild (12.5oC) and low (3oC) temperatures. Fermentation was performed by either spontaneous microbiota or *Latilactobacillus sakei* CTC494 bioprotective starter culture. Physicochemical characterization and culture dependent (MRS and MSA plates) and independent (16S rDNA sequencing) analysis were performed at day 0, 4, 12, end of ripening (a_w<0.90) and after a 15-day refrigerated storage. Temperature was the most important factor determining the change of pH, a_w and LAB levels, while the presence of starter culture affected the acidification and the time for LAB reaching the stationary phase (ca. 8.5 log cfu/g). Metataxonomic results showed that meat batter without starter culture and specially the batch containing liver ingredient showed the highest diversity due to the microbiota of this ingredient. From day 4, diversity decreased, *L. sakei* being the most abundant species (>70%) in all the batches except for that formulated without starter culture and with liver ingredient. In batches containing starter culture, *L. sakei* CTC494 clearly lead the fermentation. Sodium reduction had a low impact on the diversity of *fuet* microbiota. Conversely, absence of nitrifying agents resulted in higher microbial diversity compared with the batch with nitrite. Nutritionally improved formulations and low-temperature processes, usually used to ensure food safety of nitrite-free dry fermented sausages, only caused a minor shift on the physicochemical characteristics and bacterial communities of *fuet*.

Pòster 18

Meta-analysis of the microbial inactivation under non-thermal high pressure processing of fruit and vegetable juices and purees.

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High pressure processing (HPP) is a non-thermal preservation technology alternative to thermal pasteurization for fruit and vegetable juices and purees, with an increasing market trend thanks to its minimal effect on nutritional and organoleptic characteristics. The purpose of this study was to collect and meta-analyze available data on HPP inactivation of *Listeria* spp. *and Escherichia coli* in fruits and vegetables. From an extensive literature search, 55 articles were selected providing log10 reduction data (1284 values). Up to 12 articles provided data to estimate the D_P, as the HPP time to obtain a decimal reduction (84 values). Principal Component Analysis and Generalized Linear Mixed models were used to identify significant factors impacting on kinetic parameters (D_P), including pressure, pH category (pH<4; 4≤pH≤4.5; pH>4.5) and microorganism as fixed effects, while strain (nested to microorganism) and study as random effects. Secondary Bigelow models with the *Log* D_{P_ref} and z_P as a function of pH category and/or microorganism were fitted to the entire data set. Pressure level and pH category explained 91-93% of the data variability and the mixed models confirmed the significance of these factors, together with the microorganism. Through the global model fitting to *Log* D_P, a Bigelow-based model was obtained with Log D_{P_ref} parameter depending on pH category and a common z_P for *Listeria* and *E. coli*. When compared with the $\log_{10}$ reduction data collected from literature (not used to build the model), most of the predictions provided by the *Log* D_P model were within the acceptable simulation zone (±1 log) or fail-safe, while fail-dangerous predictions occurred only with 15% and 21% of the data for *Listeria* and *E. coli*, respectively. The global *Log* D_P model is proposed as a good conservative tool useful for risk assessment, for benchmarking and for setting the HPP conditions to comply with the performance criteria.

Pòster 19

Role of mycobacteria-polarized macrophages in bladder cancer treatment

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Macrophages play a very important role in mycobacterial infections, but also in the processes of tumor progression. The so-called tumor-associated macrophages (M2) are characterized by activating an anti-inflammatory and oncogenic response. Reversion of M2 macrophages to M1 macrophages, with inflammatory and antitumor properties, is possible due to the plasticity of macrophages. *Mycobacterium bovis* BCG (BCG) is currently used as a vaccine for tuberculosis and as an antitumor agent in patients with non-invasive non-muscular bladder cancer. Its role in the tumor microenvironment is not entirely defined.

Our study aimed to evaluate the immunomodulatory potential of BCG and *Mycolicibacterium brumae*, a non-pathogenic mycobacterium with antitumor capacities, in the phenotypic processes of polarization and reversion of M1 and M2 macrophages and their effect in the cell migration and invasion of bladder cancer cells.

Our results indicate that both BCG and *M. brumae* are capable of polarizing and reversing macrophages towards an antitumor phenotype contributing to the diminishing of the migratory and invasiveness capacities of T24 and J82 bladder cancer cells.

Pòster 20

Assessment of the presence of potentially zoonotic bacterial pathogens in fecal samples of *Pipistrellus kuhlii* in connection to four differently degraded Mediterranean ecosystems

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Bats are a highly diverse group of mammals which play a vital role in the maintenance and promotion of ecosystem health. Conversely, bats are well-known hosts of a wide range of zoonotic pathogens. The emergence of such pathogens and their spillover into livestock and human populations have increased in the last decades as a result of ecological, demographic and socio-economic changes at a global scale. Consequently, a multidisciplinary approach on the relationship between anthropogenic ecosystem degradation and zoonotic spillover risk have become paramount to prevent future pandemic events. In turn, anthropogenic factors have presumably caused a rapid decline of bat populations worldwide. Therefore, understanding the differential responses of bat species to habitat disturbance is critical for their conservation. The aim of this project was to study the link between the presence of enteric bacteria of animal and public health concern in fecal samples of insectivorous bats *dwelling in four ecosystems with different* level of environmental degradation. Fecal samples (n=32) were collected during summer 2021 directly from of Kuhl's pipistrelle *bats* (*Pipistrellus kuhlii)* captured in the following Mediterranean ecosystems: old-growth forest (n=8), extensive agriculture landscape (n=6), young forest (n=9), and urban area and intensive agriculture landscape (n=9). DNA from samples were sequenced using MinION Nanopore Oxford Technology. Differences of alpha-diversity indices between ecosystems were assessed at species-level using MicrobiomeAnalyst. The metagenomic analysis of the Operational Taxonomic Units revealed the presence of bacterial species of the Genus *Brucella, Salmonella, Acinetobacter, Bacillus, Aeromonas, Clostridium, Corynebacterium, Enterobacter, Enterococcus, Klebsiella, Listeria, Mycoplasma, Neisseria, Pasteurella, Proteus, Pseudomonas, Rickettsia, Serratia, Shigella, Staphylococcus, Streptococcus*, and *Yersinia*, with different proportions depending on the ecosystem surveyed. Statistical analysis revealed a higher richness and diversity of potentially pathogenic bacterial species in bats from extensive agriculture landscapes. The present study provides relevant insight into the relationship between the ecosystem category and the presence of potentially pathogenic bacteria in the gut microbiome of bats. However, further studies are needed to understand the specific factors that modulate those differences.

Pòster 21

The long non-coding RNA landscape of Candida yeast pathogens

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Long non-coding RNAs (lncRNAs) constitute a poorly studied class of transcripts with emerging roles in key cellular processes. Despite efforts to characterize lncRNAs across a wide range of species, these molecules remain largely unexplored in most eukaryotic microbes, including yeast pathogens of the *Candida* clade. Here, we analyze thousands of publicly available sequencing datasets to infer and characterize the lncRNA repertoires of five major *Candida* pathogens: *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida auris* and *Candida glabrata*. Our results indicate that genomes of these species encode hundreds of lncRNAs that show levels of evolutionary constraint intermediate between those of intergenic genomic regions and protein-coding genes. Despite their low sequence conservation across the studied species, some lncRNAs are syntenic and are enriched in shared sequence motifs. We find co-expression of lncRNAs with certain protein-coding transcripts, hinting at potential functional associations. Finally, we identify lncRNAs that are differentially expressed exclusively during infection of human epithelial cells for four of the studied species. Our comprehensive analyses have shed light on the non-coding genome landscapes of *Candida* pathogens paving the way for future functional characterization of the lncRNA transcripts.

Pòster 22

Longitudinal study of rectal microbiota in calves with or without diarrhea episodes before weaning

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Abstract

Background. Diarrhea in pre-weaned calves is one of the most important concerns in cattle industry worldwide. The gut microbiota is known to play a critical role in this pathology. Hence the characterization of the fecal microbiota and the differential microbial composition in health and disease can give light on the predisposition to diarrhea and help on its prevention and control in early life.

Methods. Fecal samples were taken from calves suffering an episode of diarrhea (D group) and healthy calves (H group) at 12 days of age, one month (day 33) and two months (day 61) of life. Animals from the D group were additionally sampled at the time the episode of diarrhea occurred (around day 17). The samples were processed to extract total DNA (N=7 for H group; N=6 for D group), and the 16S rRNA gene was sequenced. The sequences were bioinformatically analyzed to infer the microbial populations and find the differential taxa between groups.

Results and discussion. Calves from the H group showed an earlier microbial stabilization when compared to those in the D group. Nevertheless, neither changes in diversity nor differently abundant taxa were identified when H and D groups were compared at the same timepoints, possibly due to the high variability found between animals. Importantly, we detected differences when the core microbiota and the microbial network correlations were compared between groups, mainly among low abundant taxa. Further analysis including a higher number of animals will be useful to confirm these results.

Conclusions. The fecal microbiota of calves showed different alpha-diversity dynamics regarding the health status, which might be the reflection of changes in low-abundant microbes. The results altogether suggest that the establishment of a balanced microbiota at these early stages may be related with the health status of the animals.

Pòster 23

pETS-IBECGLOW: A new generation of bacteria promoter-probe and transposon-delivery plasmids

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Bacterial processes are conditioned by the genic expression from the microorganisms that are under specific environment condition. For that, the analysis of the different parameters which can affect the general phenotype of the bacteria is a good way to study these phenomena. Reporter genes like fluorescent proteins (FP) allowed the study of gene promoter activities and it's regulation *in vivo* or in *in vitro* models. Nowadays several FPs are used for different objectives due to the fact that they are a non-invasive tool in molecular biology. Transcriptional fusions between a promoter of interest and this FPs are a good way to measure the gene activity and it can be done in a plasmid. These are known as promoter-probe vectors, but it's use requires the presence of a selection pressure for its stability and that is usually conferred by an antibiotic addition. For that it is desirable to integrate the promoter-reporter fusions in the bacterial genome to avoid the requirement of antibiotic addition for the plasmid maintenance and integrity. Homologous recombination using suicide vectors, and the utilization of mobile elements like Tn5 transposon system are two main techniques used to insert foreign DNA fragments into the bacterial genome.
In this work, a new generation of promoter-probe and transposon-delivery vectors namely pETS-IBECGLOW were designed and developed. These vectors contain the genes encoding for different FPs, under the regulation of the three promoters of *Pseudomonas aeruginosa* ribonucleotide reductases (RNRs) classes. The plasmids are designed to improve the promotor-dependent FPs expression and preventing the non-specific background leaking.

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Pòster 24

Easily applicable modifications to electroporation conditions improve the transformation efficiency rates for rough morphotypes of fast-growing mycobacteria

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Fast- and slow-growing mycobacteria with smooth and rough morphotypes have been successfully transformed using electroporation. However, transformation efficiencies differ widely between species and strains. In this study, *Mycobacteroides abscessus* and *Mycolicibacterium brumae* were used to improve current electroporation procedures for fast-growing rough mycobacteria. The focus was on minimizing three well-known and challenging limitations: the mycobacterial restriction-modification systems, which degrade foreign DNA; clump formation of electrocompetent cells before electroporation; and electrical discharges during pulse de-livery. The different strategies herein presented successfully address these three limitations and clearly improve the electroporation efficiencies over the current procedures. The results demonstrated that, combining the developed strategies, transformation rates are clearly improved for fast-growing rough mycobacteria.

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Pòster 25

Remodelling the bladder immune microenvironment by mycobacterial species with changes in their cell envelope composition

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Bacillus Calmette–Guérin (BCG) is the most effective intravesical treatment for non-muscle invasive bladder cancer. Nevertheless, it is not always effective and frequently has undesirable side effects. Here, we characterized the bladder tumour immune microenvironment induced by a panel of mycobacteria with variation in their cell envelope composition using an orthotopic murine model of bladder cancer, to provide mechanistic insights into the properties of mycobacteria as immunomodulatory agents and to identify more effective and safer therapeutic strategies.

We provide evidence that the induced global immune microenvironment was strikingly different between the two mycobacterial species tested (BCG and *Mycobacterium brumae)*, affecting both innate and adaptive immunity. Compared with *M. brumae*, BCG triggered a more robust infiltration of $CD4^+$ and $CD8^+$ T-cells skewed toward an effector memory phenotype, with higher frequencies of neutrophils/gMDSCs and inflammatory monocytes, and higher $T_{EM}/CD4^+$ T_{reg} ratios. Conversely, *M. brumae* treatment triggered higher proportions of activated effector immune cells and reparative monocytes and lower ratios of T_{EM} cells/$CD4^+$ T_{reg}. Notably, the mycobacterial cell envelope composition in *M. brumae* had a strong impact on the tumour immune microenvironment, shaping the B-cell composition, T-cell maturation profile and myeloid compartment, thus improving the inflammatory and regulatory/suppressive balance.

Overall, we demonstrate that mycobacterial intravesical treatment modulates the bladder tissue immune microenvironment and the induced immune infiltration is species specific and shaped by mycobacterial cell envelope composition. Therefore, the global bladder tumour immune microenvironment can be remodelled, improving the quality of infiltrating immune cells and the balance between inflammatory and regulatory/suppressive responses.

Pòster 26

3D spatial organization and improved antibiotic treatment of a *Pseudomonas aeruginosa – Staphylococcus aureus* wound biofilm by nanoparticle enzyme delivery

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Chronic wounds are a serious economic and health problem worldwide and affect up to 2 million people in Europe. Chronic wounds are associated with chronic polymicrobial infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two of the most common opportunistic pathogens found. The microbial community in the wounds is embedded within a biofilm formed by the bacteria's extracellular polymeric substance (EPS). The EPS is a network made of polysaccharides, proteins, lipids, and extracellular DNA, which hinders the transport of antibiotics inside the biofilm and promotes the presence of microorganisms with antimicrobial tolerance. Due to this extreme recalcitrance, it is necessary to investigate therapies that improve the penetrability and efficacy of antibiotics.

In this context, our main objective was to study the interactions and colocalization of *P. aeruginosa* and *S. aureus* within the wound biofilm to understand their synergistic relationship. We used an optimized *in vitro* wound model that mimics an *in vivo* wound to coculture *P. aeruginosa* and *S. aureus*. Using this model, we demonstrated that antibiotic monotherapy differentially affects the two bacterial species in the mixed biofilm, driving one species to overcome the other. In contrast, dual antibiotic therapy efficiently reduces both species while maintaining a balanced population. In addition, we analyzed the effects of different biofilm dispersion strategies, such as free enzymes and enzyme-functionalized nanoparticles in combination with antimicrobial therapies. We showed that deoxyribonuclease I nanoparticle treatment has a potent antibiofilm effect, whereas α-amylase and cellulase are less effective. Finally, confocal and electronic microscopy images suggest a three-dimensional colocalization model consisting of bacterial aggregates within the biofilm structure, which could contribute to the low efficacy of antibiofilm treatments on bacteria.

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Pòster 27

Methodologies for the identification of keystone species from microbiome datasets and co-occurrence networks

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Microbial co-occurrence networks, inferred from shotgun metagenomics or metabarcoding sequencing data, are increasingly used in the literature as a proxy for microbial interactions, which are challenging to unravel. One of the relevant problems to tackle in this context is the identification of so-called “keystone species”, usually defined as those that have a significant impact on their ecosystem, disproportionate with respect to their relative abundance. In the context of the human microbiome keystone species may represent interesting targets to modulate the microbiome and promote e.g. a healthier status. However, the suitability of using microbial co-occurrence networks for keystone-species identification has been debated.

We here present our contribution to this debate, which follows a twofold approach. In the first place, we have exploited the availability of large-scale sequencing datasets defining oral or gut microbiome compositions in thousands of individuals and have developed an approach to define keystoneness indices directly from them, something that is rarely done. In the second place, we have used simulations, which enable keeping precise track of the relative impact of each species in the community, to test whether properties of the synthetic co-occurrence network, recovered both with classical structural and with novel topological data analysis approaches, indeed recapitulate keystoneness.

Pòster 28

Monitoring microbial community dynamics of a rotary drum bioreactor with immobilized *Trametes versicolor* developed for agricultural wastewater treatment

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Abstract: Nowadays, bioremediation is one of the most promising strategies for removal of pollutants in water. Several groups of organisms have been studied for their potential in biodegradation of xenobiotic compounds, however, in recent years, there has been an increasing interest in mycoremediation. White-rot fungi (WRF) are a group of lignin-degrading basidiomycetes that show a great potential in this regard, due to their powerful enzymatic machinery and high tolerance to pollutants. Moreover, accompanying microbial populations may interact with these fungi and accordingly may cause an impact upon removal.

In this study, the microbial communities inside a non-sterile "rotary drum bioreactor" (RDB), developed for treatment of pesticide-polluted agricultural wastewater were monitored. The white-rot fungus *Trametes versicolor* was immobilized in wood chips inside the reactor, acting as the main bioremediation agent. Microbial community dynamics were assessed by denaturing gradient gel electrophoresis (DGGE), followed by recovery and sequencing of prominent bands. These analyses showed that *T. versicolor* constituted most of the fungal immobilized biomass in the reactor, revealing its successful colonization and endurance along the experiment. Moreover, *Kosakonia*, *Novosphingobium* and *Burkholderia* were the predominant members of the bacterial assemblage present in the studied solid substrate. Pesticide removal also achieved satisfactory levels: up to 54 % removal of diuron and 48 % of bentazon after the 225-day treatment.

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