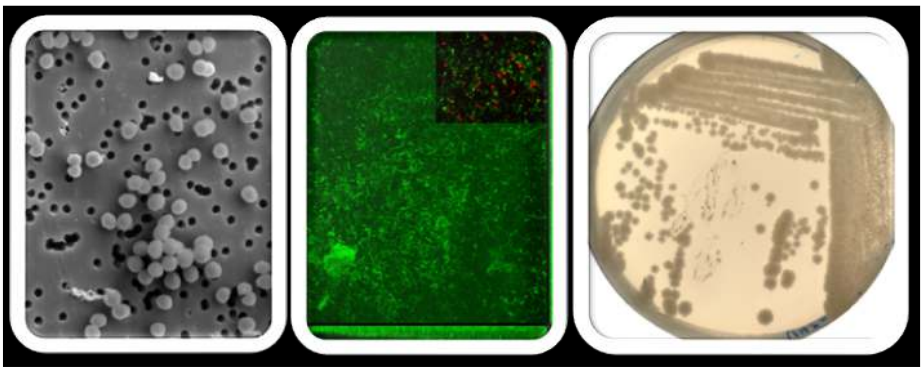
II Jornada de Microbiologia

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

29 de Novembre de 2019

Societat Catalana de BIOLOGIA

II Jornada de Microbiologia

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

PROGRAMA

Comitè Organitzador:

Eduard Torrents Serra
Institut de Bioenginyeria de Catalunya (IBEC)

Maria del Mar Cendra Gascón
Institut de Bioenginyeria de Catalunya (IBEC)

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Sponsor de la Jornada:

BioLab

Fotos portada: IBEC-BIAT group©

Divendres, 29 de Novembre de 2019

09:00 - 09:10 Recollida de documentació

Sessió MATÍ

Moderador: Susana Merino, UB, Barcelona (IBEC)

09:10 - 9:45 ***CONFERÈNCIA INAUGURAL***
Mobility of bacterial genomes using phages: the new era of transduction.
Maite Muniesa. Departament de Genètica, Microbiologia i Estadística. UB. Barcelona

09:45 – 10:00 *Origin of the mobile DHPS genes conferring sulfonamide resistance*
Miquel Sánchez-Osuna. Departament de Genètica i de Microbiologia**.** UAB.

10:00 – 10:15 *Identification of two Plesiomonas shigelloides proteins involved in flagellin glycosylation.*
Elena de Mendoza. Departament de Genètica, Microbiologia i Estadística. UB. Barcelona.

10.15 – 10:30 *Role of motility in the Ralstonia solanacearum pathogenicity.*
Jordi Corral. Departament de Genètica i de Microbiologia. UAB. Barcelona.

10.30 – 10:45 *Exploring the relationship between Quorum sensing and antibiotic resistance in Stenotrophomonas maltophilia: Opportunities for combination therapy strategies.*
Daniel Yero. Institut de Biotecnologia i Biomedicina. UAB. Barcelona.

10:45 – 11:00 *Optimal environmental and culture conditions allow the in vitro coexistence of Pseudomonas aeruginosa and Staphylococcus aures in stable biofilms.*
Maria del Mar Cendra. Grup Infeccions Bacterianes i Teràpies Antimicrobianes. IBEC. Barcelona.

11:00 – 11:30 Sessió de pòsters – Coffee break

Moderador: Margarita Martínez Medina. Universitat de Girona.

11:30 – 11:45 *Extracellular vesicles released by gut microbiota modulate host immune responses throught specific activation of dentritic cells.*
Natalia Diaz-Garrido. Departament de Bioquímica i Fisiologia**.** UB. Barcelona.

11:45 – 12:00 *Diversity of Penicillium species in River Sediments from Catalonia.*
Daniel Torres. Unitat de Micologia. URV. Tarragona.

12:00 – 12:15 *What's in irrigation water?: Metagenomic analysis and index microorganisms.*

Rusiñol, M. Departament de Genètica, Microbiologia i Estadística. UB. Barcelona.

12:15 – 12:30 *Yeast humanization has allowed the identification of GMP synthase as a new partner for human Glrx3 and yest Grx3/Grx4, coverging in the regulation of the ISR pathway.*
Nuria Pujol-Carrion. Departament de Ciències Mèdiques Bàsiques. UdL. Lleida.

12:30 – 12:45 *Salmonella inactivation in traditional dry-fermented sausages. A decision-supporting tool to design a corrective storage as risk mitigation strategy.*
Cristina Serra-Castelló. Food Safety Programme**.** IRTA Monells. Girona**.**

12:45 – 13:00 *Do we eat resistances? Antibiotic resitant bacteria detection in ready-to-eat foods.*
Carrillo, A. Facultat de Veterinària. UAB. Barcelona.

13:00 – 13:15 *Discussió / Millora de les Jornades de Microbiologia de la SCB.*

13:15 – 14:45 **Sessió de pòsters - Dinar**

Sessió TARDA

Moderador: Virginia Aragón (CRESA, IRTA-UAB).

14:45 – 15.15. ***CONFERÈNCIA CONVIDADA***
The rise and spread of the Acinetobacter gang!
Ignasi Roca, ISGlobal, Hospital Clínic. UB. Barcelona.

15:15 – 15:30 *Effect of ceftiofur treatment on the microbiota of pregnant sows and their offspring.*
Florencia Correa-Fiz. CRESA, IRTA-UAB. Barcelona.

15:30 – 15:45 *Prevalence and antimicrobial resistance profile of extended-spectrum beta-lactamases (ESBL) and colistin resitance (mcr) genes in Escherichia coli strains isolated from swine between 1999 and 2018 in Spain.*
Laia Aguirre. CRESA, IRTA-UAB. Barcelona.

15:45 – 16:00 *Antimicrobial resistance profil·les and characterization of Escherichia coli strains from cases of neonatal diarrea in Spanish pig farms.*
Anna Vidal. CRESA, IRTA-UAB. Barcelona.

16:00 – 16:15 *Yellow-legged gulls (Laurus michahellis) and Audouin's gull (Laurus audouinii) from Barcelona as a source of Campylobacter of public Health relevance.*
Alicia Manzanares. CRESA, IRTA-UAB. Barcelona.

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

Moderador: Maria del Mar Cendra (IBEC, Barcelona).

16:45 – 17:00 *Microbial toxin-based nanoparticles as targeted antitumoral drugs.*
Laura Sánchez-García. Institut de Biotecnologia i Biomedicina. UAB. Barcelona.

17:00 – 17:15 *Antimicrobial peptide-containing nanoparticles as therapeutics for infectious mastitis treatment.*
Jose Vicente Carratalá. Institut de Biotecnologia i Biomedicina. UAB. Barcelona.

17:15 – 17:30 *Bacterial cellulose matrices to develop enzymatically actice paper-based nanocomposites.*
Carolina Buruaga-Ramiro. Departament de Genètica, Microbiologia i Estadística. UB. Barcelona.

17:30 – 17:45 *Antimicrobial potential of host defense peptides produced as soluble nanoclusters-based recombinanat proteins.*
A. López-Caño. IRTA. Caldes de Montbui. Barcelona.

17.45 – 18.00 *Bacterial Cellulose-chitosan nanocomposites with antimicrobial and antioxidant activity.*
L. Veronica Cabañas-Romero. Departament de Genètica, Microbiologia i Estadística. UB.

18:00 – 18:15 Cloenda.
Eduard Torrents. Secció de Microbiologia, Societat Catalana Biologia, IEC

PÒSTERS

1. *Study of evolutive adaptation to Tuberculosis in a Drosophila model*. **Marta Arch**, Marte Dragset, Esther Fuentes, Pere-Joan Cardona. Experimental Tuberculosis Unit (UTE). Fundació Institut Germans Trias i Pujol (IGTP).

2. *Modifications in flagella glycosylation affect TLR5 recognition.* **Genoveva Arques**, Jorge Sangüesa, Juan Tomás, Susana Merino. Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain.

3. *Production of recombinant mammary serum amyloid A3 and study of its immunomodulatory capability.* **R. Baltà-Foix, F. Fàbregas**, L. Gifre-Renom, E. Garcia-Fruitós and A. Arís. Department of Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), 08140 Caldes de Montbui, Spain.

4. *Safety and toxicity study of the immunomodulatory agent Mycobacterium brumae in two different animal models.* **Marc Bach-Griera**, Victor Campo-Pérez, Sara Traserra, Sandra Barbosa, Laura Moya-Andérico, Sandra Guallar-Garrido, Paula Herrero-Abadía, Marina Luquin, Eduard Torrents, Esther Julián. Mycobacteria Research Lab, Department of Genetics and Microbiology, Facultat de Biociències, Universitat Autònoma de Barcelona, Building C, 08193 Bellaterra, Barcelona, Spain.

5. *Novel Oleanolic and Maslinic Acids derivatives as a promising treatment against bacterial biofilm in nosocomial infections: An in vitro and in vivo study.* **Núria Blanco-Cabra,** Karina Vega-Granados, Laura Moya-Andérico, Marija Vukomanovic, Andrés Parra, Luis Álvarez de Cienfuegos, Eduard Torrents. Bacterial Infections: Antimicrobial Therapies, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

6. *Comparative transcriptomics to identify gene expression differences between AIEC and non-AIEC isolates during in vitro cell infection.* **Queralt Bonet-Rossinyol**, Carla Camprubí-Font, Margarita Martinez-Medina. Laboratory of molecular microbiology, Biology department, Universitat de Girona, Girona, Spain.

7. *Ultrastructural external and internal analysis of Mycobacterium brumae grown on different culture media.* **Víctor Campo-Pérez**, Sandra Guallar-Garrido, Alejandro Sánchez-Chardi, Marina Luquin, Esther Julián. Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona (UAB), Bellaterra (Barcelona), Spain.

8. *Genetic diversity and virulence potential of Campylobacter coli and Campylobacter lanienae carried by wild boars from the metropolitan area of Barcelona.* Marta Marín-Sala, Teresa Ayats, Raquel Castillo-Contreras, Gregorio Mentaberre, Jorge Ramón López-Olvera, **Marta Cerdà-Cuéllar**. Centre de Recerca en Sanitat Animal, IRTA-CReSA, Campus Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain.

9. *Evaluation of the immune response in tuberculosis patients before and after receiving therapeutical surgery in Georgia*. **Albert Despuig**, Asimakis Avramopoulos, Zaira García,

Eric García, Sergo Vashakidze, Shota Gogishvili, Keti Nikolaishvili, Natalia Shubladze, Jordi Casanovas, Albert Obiols, Tomàs Aluja, Maria-Rosa Sarrias, Cristina Vilaplana. Experimental Tuberculosis Unit (UTE). Fundació Institut Germans Trias i Pujol (IGTP).

10. *Differences in the tumor proliferation inhibitory ability among strains of the same mycobacterium species.* **Paula Herrero-Abadía**, María Muñoz, Marina Luquin, Esther Julián. Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona (UAB), Bellaterra (Barcelona), Spain.

11. *Estudi i avaluació de nous probiòtics.* **Yuste Aida**, Arosemena L, Nogué E, Calvo Mª Àngels. Grup de Recerca en Microbiologia Aplicada i Mediambiental, Facultat de Veterinària. Universitat Autonòma de Barcelona.

12. *Dynamics of Campylobacter infection in a colony of yellow-legged gulls (L. michahellis).* **David Narváez** , Raül Ramos, Teresa Ayats, Marta Cerdà-Cuéllar. Centre de Recerca en Sanitat Animal, IRTA-CReSA, Campus Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain.

13. *Biodistribution and transcytosis of liposome-encapsulated bacteriophages in oral phage therapy.* **Jennifer Otero**, Alba García-Rodríguez, Mary Cano-Sarabia, Daniel Maspoch, Ricard Marcos, Pilar Cortés, Montserrat Llagostera. Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain.

14. *Mechanism of action of NrdR, a system confined to bacteria behind the control of all dNTP synthesis.* **Lucas Pedraz**, Arkadiusz Szura, Maria Solà, Eduard Torrents. Bacterial Infections: Antimicrobial Therapies (BIAT), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

15. *Bottom-Up Instructive Quality Control in the Biofabrication of Smart Protein Materials*. **Naroa Serna**, Fabián Rueda, María Virtudes Céspedes, Oscar Conchillo-Solé, Alejandro Sánchez-Chardi, Joaquin Seras-Franzoso, Rafael Cubarsi, Alberto Gallardo, Mireia Pesarrodona, Neus Ferrer-Miralles, Xavier Daura, Esther Vázquez, Elena García-Fruitós, Ramón Mangues, Ugutz Unzueta, and Antonio Villaverde. Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, 08193, Cerdanyola del Vallès, Spain.

16. *High Time Resolution and High Signal-to-Noise Monitoring of the Bacterial Growth Kinetics in the Presence of Plasmonic Nanoparticles*. Marija Vukomanovic, Eduard Torrents. Bacterial Infections and Antimicrobial Therapies (BIAT), Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

17. *Prevalence of carbapenem and CR genes in stool samples of wild boars of Barcelona region.* Seminati C., **Vidal A**., Conejero C, Mentaberre G, López-Olvera J.R., Darwich L. Departament de Sanitat i Anatomia Animal, Universitat Autònoma de Barcelona (UAB), Cerdanyola del Vallès, Spain.

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COMUNICACIONS

Mobility of bacterial genomes using phages; the new era of transduction

Maite Muniesa[1], Pedro Blanco-Picazo[1], Daniel Toribio Avedillo, Lorena Rodríguez

[1] Microbiology, Virology and Biotechnology section Department of Genetics, Microbiology and Statistics Faculty of Biology. University of Barcelona Diagonal 643. Prevosti building. Floor 0, 08028 Barcelona. Spain

Bacterial chromosome features numerous prophages, many of which encode virulence-related genes, providing some advantage for their bacterial host. In contrast, other prophages do not encode any known beneficial gene, are unable to generate virions (cryptic phages) or have lost part of their genome, (remnants or ancient prophages). The issue of why these remnant sequences prevail in the bacterial chromosome is intriguing. If the information these fragments contain is incomplete, and bacterial cells tend to eliminate any information not directly beneficial for their adaptation, why do they accumulate so many prophages?

Using *Escherichia coli* as a model and focusing on antibiotic resistance genes (ARG), we observe that *E. coli* generate phage particles containing ARG as a part of a mechanism that bacteria use to spread its own DNA. Some examples of other bacterial genera presented and new mechanisms of transduction described also supports the idea that phages are not only “enemies” of the bacterial cells, but prophages in bacterial chromosomes are in fact used by bacteria as a mechanism to disseminate their own genetic content.

Origin of the mobile DHPS genes conferring sulfonamide resistance

Miquel Sánchez-Osuna[1], Pilar Cortés[1], Jordi Barbé[1], Ivan Erill[2]

[1]Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona
[2]Department of Biological Sciences, University of Maryland Baltimore County

Resistance to antibiotics and chemotherapeutic agents can be acquired through mutation or via the acquisition of resistance determinants through lateral gene transfer. It is widely accepted that antibiotic resistance genes originate from homologs in either the microbes that naturally produce the antibiotics or their competitors, and that the commercial introduction of antibiotics set the stage for the rapid selection and proliferation of resistant strains.

The origin of resistance against chemotherapeutic agents, such as sulfonamides, is harder to elucidate. Since these were designed *in vitro*, it seems unlikely that genes conferring resistance existed before their introduction. Sulfonamides are chemotherapeutic agents that act as inhibitors of the di-hydro-pteroate synthase (encoded by *folP* gene), leading to growth arrest. Sulfonamides were developed in the 1930's, and resistance via chromosomal mutations was reported in the 1940's. In the early 1970's, plasmid-borne *sul* genes encoding alternative sulfonamide-resistant DHPS enzymes were described.

The presence of a Sul motif associated with *sul*-encoded proteins in chromosomal *folP* and the results of molecular Bayesian phylogeny, indicated that the chromosomal origin of the clinical *sul* genes were the *Rhodobiaceae* and the *Leptospiraceae* families. Broth microdilution revealed that these chromosomally-encoded *folP* genes confer resistance to sulfonamides. These results indicate that the emergence of the Sul motif in chromosomally encoded FolP proteins is ancient and considerably predates the clinical introduction of sulfonamides. Thus, genes conferring resistance to synthetic chemotherapeutic antimicrobials are available in the microbial pangenome, and its dissemination can take place in short time upon the clinical introduction of these synthetic compounds.

Identification of two *Plesiomonas shigelloides* proteins involved in flagellin glycosylation

Susana Merino[1,2] Elena de Mendoza[1,2] Kelly M. Fulton[3] Susan M. Twine[3] and Juan M. Tomás[1,2]

[1] Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain.
[2] INSA-UB (Institut de Recerca en Nutrició i Seguretat Alimentària), Barcelona, Spain.
[3] National Research Council, Ottawa, ON, Canada.

In *Plesiomonas shigelloides*, motility is mediated by lophotrichous flagella when grown in liquid medium. However, *P. shigelloides* 302-73 strain also shows lateral flagella when grown in solid or semisolid media, being the only member of the *Enterobacteriaceae* family able to build inducible lateral flagella. In *P. shigelloides* 302-73 strain, both flagella types are glycosylated by a legionaminic acid (Leg) derivative, and genes involved in the Leg pathway are clustered between the two regions involved in the biosynthesis of lophotrichous flagella. Genes involved in lateral flagella biosynthesis, on the other hand, are clustered in a unique chromosomal region. Flagellin O-glycosylation is essential for bacterial flagella formation, either polar or lateral, as gene mutants on the Leg biosynthetic pathway result in non-flagellated bacteria. Moreover, genes encoding orthologous proteins of *Campylobacter jejuni* motility accessory factor (Maf) proteins were found adjacent to the Leg biosynthetic genes (maf-1) and upstream of the lateral flagellin, lafA (maf-5). Phenotypic analysis of mutants generated in these two maf genes showed that the maf-1 mutant lacks lophotrichous flagella and has no effect in lateral flagella, while the maf-5 mutant lacks lateral flagella and has no effect in lophotrichous flagella. *P. shigelloides* 302-73 is the first member of the *Enterobacteriaceae* family reported to be O-glycosylated by a Leg derivative in both polar and lateral flagella, and the presence of both the lateral flagella cluster and the Leg O-flagella glycosylation genes seem to be widely spread features among the *P. shigelloides* strains tested.

Role of motility in the *Ralstonia solanacearum* pathogenicity

Jordi Corral[1], Pau Sebastià[2], Núria S. Coll[2], Jordi Barbé[1], Jesús Aranda[1] and Marc Valls

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[2]Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra 08193, Cerdanyola del Vallès (Barcelona), Catalonia, Spain

Ralstonia solanacearum is a bacterial plant pathogen responsible of bacterial wilting disease, which produce important economic losses in agricultural sector worldwide. To infect and colonize different plant tissues through the vascular system, *R. solanacearum* uses two types of movement strategies. The first one is the swimming motility, an individual cell movement powered by rotating flagella and produced in aqueous environments. The second is the twitching motility, a coordinated multicellular movement driven by the extension, attachment and retraction of the type IV pili (TFP) appendages in solid surfaces or viscous media.

In *R. solanacearum*, the implication of chemotaxis in plant colonization through flagella regulation has previously reported, specifically by CheW and CheA proteins, which enable bacterial cells to move towards more favorable conditions in function of sensing specific chemicals. In this study, we have identified homologs to the PilI (CheW-like) and ChpA (CheA-like) proteins of *Pseudomonas aeruginosa*, in which it has been suggested that both proteins play a role in TFP-associated motility regulation.

In the present work, we demonstrate that the *pilI* and *chpA* genes of *R. solanacearum* are implicated in twitching motility and other related virulence factors. Furthermore, this work provides evidences of a cross-effect in both kinds of movements. Finally, we also demonstrate that genes encoding the major pilin (PilA) and the flagellin (FliC) subunits are required for *R. solanacearum* full virulence.

Exploring the relationship between *Quorum sensing* and antibiotic resistance in *Stenotrophomonas maltophilia*: Opportunities for combination therapy strategies.

Daniel Yero[1], Pol Huedo[1], Xavier Coves[1], Oscar Conchillo-Solé[1], Xavier Daura[1,2], Isidre Gibert[1]

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[2] Catalan Institution for Research and Advanced Studies, Barcelona, Spain.

Healthcare-associated infections caused mainly by multidrug-resistant bacteria are a serious problem worldwide. *Stenotrophomonas maltophilia* has recently become an important nosocomial pathogen particularly affecting immunocompromised and cystic fibrosis patients. The major concern with this bacterium is because it is intrinsically resistant to multiple antibiotics, and it acquires new resistances very quickly. The quorum sensing (QS) system represents an important virulence factor in *S. maltophilia*, governing biofilm formation and cell motilities. Biofilm production allows these bacteria to become inaccessible to antimicrobials. Antibiotic resistance and QS have been explored independently in *S. maltophilia*. However, knowledge about the connection between both processes is still limited. QS in *S. maltophilia* is mainly based on a fatty acid known as DSF (diffusible signal factor), which is synthesized and detected by proteins encoded on the *rpf* gene cluster. Two variants of the *rpf* cluster (*rpf*-1 and *rpf*-2) distinguish two groups of phenotypically different strains of *S. maltophilia*. We have demonstrated, in a group of genotypically different clinical isolates, that strains carrying the *rpf*-2 cluster, besides being more virulent, are in general more resistant to the last resort antibiotic colistin. On the other hand, *rpf*-1 strains are significantly more resistant to beta-lactams. Furthermore, whole-transcriptome sequencing analysis under conditions promoting QS activation revealed up-regulation of genes coding for proteins belonging to, among others, the beta-lactamase and drug/metabolite transporter families. Altogether, these results give us clues about a possible connection between QS and resistance. In addition, we have recently shown than a combination therapy using a DSF analogue and colistin could be effective in eliminating biofilm by pathogens such as *S. maltophilia* and could reduce the effective dose of the antibiotic. Overall, targeting the mechanism of QS in *S. maltophilia* seems to be an alternative to conventional antibiotic treatments and a strategy that would mitigate the increase of resistance mechanisms.

Optimal environmental and culture conditions allow the *in vitro* coexistence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in stable biofilms

Maria del Mar Cendra, Núria Blanco-Cabra, Lucas Pedraz and Eduard Torrents

Bacterial Infections: Antimicrobial Therapies lab (BIAT), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain

Most chronic infections occur due to the inherent capacity of some bacterial pathogens to grow in biofilms; and there is evidence that most of these biofilms are composed by multiple microbial species growing together. Polymicrobial infections challenge the antimicrobial chemotherapy to administrate and often aggravates the disease's outcome. However, the coexistence between species that occurs in some infections remains hard to achieve *in vitro* since bacterial fitness differences eventually lead to a single organism dominating the mixed culture. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are major pathogens found growing together in biofilms in disease-affected lungs or wounds.

In this study, we aimed to find optimal environmental and culture conditions to grow *P. aeruginosa* and *S. aureus* together in mixed biofilms *in vitro*, including stable populations of both microbes. After testing different culture media, additives and environmental parameters we have designed a combination of conditions that allow *P. aeruginosa* and *S. aureus* to grow in mixed biofilms during, at least, three days over different abiotic surfaces under static incubation as well as in continuous-flow. The culture conditions unravelled in this study allow the stable formation of separate *S. aureus* microcolonies that grow embedded in a *P. aeruginosa* biofilm, as well as *S. aureus* biofilm overgrowth when a valuable additive is added to the system, in an environment that dampens the pH rise. Additionally, we have detected a very marked oxygen stratification in the co-culture system that seriously compromises *Staphylococcus* viability and modulates the bacterial growth and distribution in the mixed biofilm. To validate the combination of the coculture conditions and environmental prerequisites identified, we treated the mixed biofilms with known antibiotics to confirm differences in antibiotic tolerance depending on whether the strains were growing in mono- or coculture biofilms.

This work was supported in part through grants to E.T.S. from the Ministerio de Ciencia, Innovación y Universidades (BIO2015-63557-R and RTI2018-098573-B-100), Generalitat de Catalunya (2017 SGR1079), La Caixa Foundation and Catalan and Spanish Cystic Fibrosis foundations.

Extracellular vesicles released by gut microbiota modulate host immune responses through specific activation of dendritic cells

Natalia Diaz-Garrido[1], María-José Fábrega[1,2], Rodrigo Vera[1], Marta Riera[1], Rosa Giménez[1], Josefa Badia[1] and Laura Baldomà[1].

[1]Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona; Institut de Biomedicina de la Universitat de Barcelona - Institut de Recerca Sant Joan de Déu, Spain.
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Gut microbiota establishes reciprocal relationships with the host intestinal epithelium and immune system. Bacterial membrane vesicles (MVs) are key players in microbiota-host communication. Dendritic cells (DCs) in the lamina propria play a main role in sampling gut microbes and shaping appropriate immune responses that allow to preserve intestinal homeostasis. The objective of this study was to evaluate the immunomodulatory properties of the MVs from the probiotic strain E. coli Nissle 1917 (EcN) in terms of DC-derived adaptative immune responses and compare the effects with those elicited by commensals *E. coli*.

To this end, MVs were isolated from the probiotic EcN, an EcN derived mutant lacking the polysaccharide capsule (EcN:K5) and the commensal strains ECOR12, ECOR63 and ECOR53. Monocytes isolated from buffy coats from healthy donors were cultured and differentiated into DCs for 7 days, and then incubated with MVs for 24 h. Stimulated DCs were then co-cultured with isolated CD4+ T cells. The ability of MVs to specifically activate DCs and subsequent Th-type responses was evaluated by cytokine quantification and flow cytometry analysis of specific markers.

Results showed that EcN MVs induce intricated Th1/Th17/Th22/Treg responses consistent with the beneficial effects of this probiotic. Th17/Th22 responses were common to commensal *E. coli*-derived vesicles but specific differences were observed for Th1 and Treg responses. ECOR12 MVs increase the Treg/Th17 balance and active Th22 response but do not trigger appropriate Th1 responses.

This work provide evidence that microbiota MVs are sensed by immature DCs and specifically modulate T cell responses, thus acting as key players in the modulation of the host immune system. Probiotic-derived MVs could be explored as a safe (bacteria free) strategy to develop new functional food ingredients targeting gut microbiota balance or intestinal inflammation.

Diversity of *Penicillium* species in River Sediments from Catalonia

Daniel Torres, Josepa Gené, Dania García

Mycology Unit, Medical School and IISPV, Universitat Rovira i Virgili, Reus, Tarragona, Spain

Penicillium is a common and ubiquitous genus, which species diversity has extensively been studied from outdoor and indoor environments. However, a little is known about the spectrum of *Penicillium* species inhabiting river sediments. Although, previous studies of our research group indicated that this substrate constitutes a great reservoir of microfungi, the diversity of this genus in this substrate has poorly been studied. Therefore, the aim of this study was to determine the diversity of penicillia-fungi in river sediments from Catalonia and to characterize the new species if they were detected. We selected streams or rivers placed in natural areas from Barcelona and Tarragona, and a total of 21 samples were collected and studied using different cultive-dependent isolation methods. We used sequence analysis of the β-tubulin (*BenA*) gene to identify a total of 95 isolates, which were distributed in 30 known *Penicillium* species. The most represented section of *Penicillium* in our samples was *Citrina*, with 39 isolates representing 11 species, followed by *Brevicompacta* and *Fasciculata*, with 14 and 11 isolates, respectively, but with only two species in each of those sections. The most frequent species isolated were *P. brevicompactum* (n = 12), *P. crustosum* (n = 11) and *P. pancosmium* (n = 10). However, five *Penicillium* isolates could not be assigned to any particular species. We resolved the taxonomy of some of these isolates through a multi-locus sequences analysis including the ITS regions, fragments of the *BenA*, calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) genes, simultaneously with a detailed phenotypic study. As a result, at least two new species are proposed, *P. ausonae* and *P. guarroi.*

WHAT'S IN IRRIGATION WATER?: METAGENOMIC ANALYSIS AND INDEX MICROORGANISMS

Rusiñol M.[1], Martínez-Puchol S.[1], Hundesa A.[1], Timoneda N.[1,2], Fernández-Cassi X.[1], Pérez-Cataluña A.[3], Fernández-Bravo A.[3], Moreno-Mesonero L.[4], Moreno Y.[4], Alonso J.L.[4], Figueras M.J.[3], Abril J.F.[2], Bofill-Mas S.[1], Girones R[1]

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Agriculture irrigation is increasing the total annual water use in Europe. Reclaimed water is a good alternative to reduce the dependence of conventional irrigation sources, but it is also a challenge as pathogen exposure may increase. Viruses (e.g., noroviruses and hepatitis A and E virus), bacteria (e.g., Salmonella spp. and pathogenic *Escherichia coli*) and protozoa (e.g., Cryptosporidium parvum, Giardia intestinalis) are well-known contributors to reported food-borne illnesses linked to contaminated fresh produce, however it is known that the number of food-borne infections is underestimated and the list of pathogens uncompleted.

Within the Metawater project (Water JPI), the viral, bacterial and protozoan populations were monitored over one year period in different irrigation water sources (reclaimed water, groundwater and river water) by a metagenomic approach. The occurrence of a set of viruses, bacteria and protozoa, was also quantified after using a single concentration method for all microorganisms tested.

Most of the known viral species (>77%) derived from plants and bacteriophages. The viral diversity in the river water shifted over seasons, increasing bacteriophage reads during autumn and winter, when Noroviruses GII were also detected. Emerging human pathogens as HEV and Naegleria fowleri were detected in groundwater during the summer sampling. A wetland used as a sustainable system, to treat secondary effluents from urban WWTP and produce reclaimed water, seems to restore the natural microbial community as the virome and bacteriome resemble those present in river water samples. If reclaimed water will be used as a source of irrigation, the analysis of some potential pathogens, like HAdV during the peak irrigation period (summer and spring) or NoV during the coldest months, could complement and enrich existing water management strategies.

Yeast humanization has allowed the identification of GMP synthase as a new partner for human Glrx3 and yeast Grx3/Grx4, converging in the regulation of the ISR pathway

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The human monothiol glutaredoxin Glrx3 (PICOT) is ubiquitously distributed in cytoplasm and nuclei in mammalian cells. Its overexpression has been associated to the development of several types of tumors whereas its deficiency might cause retardation in embryogenesis. Its exact biological role has not been well resolved, although a function as chaperone distributing iron/sulphur clusters is currently accepted. Yeast humanization and the use of a mouse library has allowed us to find a new partner for PICOT: the human GMP synthase (hGMPs), both proteins carry out collaborative functions regarding down-regulation of the S. cerevisiae ISR pathway, in conditions of nutritional stress. Glrx3/hGMPs interact through conserved residues which bridge iron/sulphur clusters and glutathione. This mechanism is also conserved in budding yeast whose proteins Grx3/Grx4 along with GUA1 also downregulate ISR pathway. Heterologous expression of Glrx3/hGMPs efficiently complements Grx3/Grx4 for these functions. Moreover, heterologous expression of Glrx3 efficiently complements the novel participation in chronological life span that has been characterized for both Grx3 and Grx4 in this study. Our results underscore that the family Glrx3/Grx3/Grx4 present an evolutive and functional conservation in specific signaling events than contribute to cell life extension.

Salmonella inactivation in traditional dry-fermented sausages. A decision-supporting tool to design a corrective storage as risk mitigation strategy

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The zero tolerance for *Salmonella* in ready-to-eat food poses a challenge for the meat industry, particularly in shelf-stable dry-fermented sausages (DFS). In this work, the behaviour of *Salmonella* in the traditional Catalan acid and low-acid DFS (*fuet*) was studied as a function of a_w and storage temperature. The final aim was to build a decision supporting tool to set a corrective measure leading to a safer product.

The survival of *Salmonella* during the storage of acid (with starter culture) and low-acid (without starter culture) DFS was evaluated through challenge testing. *Salmonella* was inoculated in the meat batter at 6 Log cfu/g for manufacture of DFS with different a_w (0.88, 0.90 and 0.93). DFS storage was performed at 4, 8, 15 and 25 ºC. The survival of *Salmonella* was periodically monitored on chromogenic agar. The Weibull model was fitted to Log counts data to estimate inactivation kinetic parameters. The impact of temperature and a_w was evaluated using a polynomial approach.

Salmonella viability decreased during storage of DFS and the Weibull model satisfactorily fitted the data. The effect of a_w and temperature was statistically significant. Decreasing a_w and increasing temperature caused a decrease of the time for the first Log reduction of *Salmonella* (δ). A common shape inactivation curve (p) was obtained for acid and low-acid DFS, while δ was product specific, indicating that starter culture affected the time required for the first Log reduction. Interestingly, a correlation was observed between the δ values obtained for low-acid and acid DFS: the time to 1 Log-inactivation in acid sausages was 50% (4 ºC) to 21% (25 ºC) shorter than in low-acid sausages.

Based on technologically and commercially feasible time-temperature corrective storage periods, the developed models can be used to define post-lethality treatments to enhance *Salmonella* inactivation and improve DFS safety.

Do we eat resistances? Antibiotic resistant bacteria detection in ready-to-eat foods.

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The increase of antibiotic resistant bacteria is a menace for public health and they are different routes of propagation that play a role in it, such as the food chain. At the moment, the EU executes surveillance programs on raw foods of animal origin. Diverse ready-to-eat products have been studied in order to assess their resistances and its inference on public health. In this experiment, 16 samples from 4 different food groups (animal and plant origin) have been collected from assorted food establishments in the province of Barcelona (Spain). A number of pathogenic and hygiene indicator microorganisms have been isolated and tested for antibiotic susceptibilities (by disk diffusion method). The results revealed that 7,3% showed resistances and 6,3% susceptibility, but only by exposition to higher doses than recommended. In addition, 56% of foodstuffs carried at least one antibiotic resistant bacterium and resistances were identified in all analyzed groups. The possibility of resistance transfer between bacteria is a known mechanism, something that was confirmed by our results (as all resistances were acquired). Ready to eat food consumption is currently increasing and the fact that antibiotic resistant bacteria were detected shows a worrying trend. It can therefore be concluded that it is a risk to public health.

The rise and spread of the *Acinetobacter* gang!

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During the last decades we have witnessed the emergence and rapid dissemination of antimicrobial resistance among common bacterial pathogens, such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, to name a few, but the global spread and acquisition of resistance to multiple antimicrobial agents of *Acinetobacter baumannii* and related species have not received that much attention. The recent improvements in molecular methods as well as mass spectrometry have revealed the presence of major multidrug resistant (MDR) clonal lineages of *A. baumannii* all over the planet as well as an increase in the number of isolates from related species that are being recovered from human samples, which may pose a health challenge if neglected. In this talk we will discuss the resistance profiles and worldwide distribution of established international clones as well as the potential emergence of novel human pathogens within the *A. baumannii* group.

Effect of ceftiofur treatment on the microbiota of pregnant sows and their offspring

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The view on antimicrobials has changed due to the increased knowledge on the importance of microbiota composition in different niches. Antimicrobials can no longer be considered only beneficial, but also potentially deleterious for favorable bacterial populations. Antimicrobials are used to treat or prevent infections and, sometimes in sows, to reduce pathogen transfer to the piglets. The goal of this study was to characterize the effect of ceftiofur in pregnant sows on their vaginal and nasal microbiota, together with the concomitant putative effect on the nasal microbiota of their litters.

Nine sows were housed in two different rooms. Five sows were treated with ceftiofur injected 3-6 days before farrowing, while sows in the other room remained non-treated (n=4). Nasal and vaginal swabs were collected from the sows at farrowing, and nasal swabs were collected from piglets from different litters at birth. Vaginal (4 non-treated and 5 treated) and six nasal (3 per group) swabs from the sows, together with ten nasal swabs (5 from each sow group) from piglets were selected to study the microbiota composition. Total DNA was extracted from the swabs and submitted for NGS 16S rDNA gene sequencing.

Beta diversity analysis showed that the nasal microbiota from the piglets resembled more the nasal microbiota than the vaginal microbiota from the sows, although with differences. Noteworthy, *Caulobacter* in the treated group (7.1-67.97%) and *Enhydrobacter* in the non-treated group (9.2%-67.3%) were the most relative abundant genera in piglets' noses, while they were relatively low in abundance in the nasal cavity and vagina of the sows. The ceftiofur treatment did not significantly change the alpha diversity of the nasal or vaginal microbiota in sows. However, in the nasal microbiota of piglets the diversity decreased significantly, indicating a potential detrimental effect of the antibiotic applied to the sows.

Prevalence and antimicrobial resistance profile of extended-spectrum beta-lactamases (ESBL) and colistin resistance (*mcr*) genes in *Escherichia coli* strains isolated from swine between 1999 and 2018 in Spain

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The frequent usage of antibiotics in livestock has brought to the spread of resistant bacteria within animals and their products, with a global warning in public health and veterinarians to monitor such resistances. This study aimed to determine antibiotic resistance patterns and prevalence of resistance genes in pig farms during the last twenty years. Susceptibility to six antibiotics commonly used in pig production was tested by qualitative (disk diffusion) and quantitative (minimum inhibitory concentration, MIC) methods in 91 strains of *Escherichia coli* isolated between 1999 and 2018 from Spanish pig farms. Results showed resistance around 100 % for amoxicillin and tetracycline since 1999, and around 50 % for enrofloxacin. A progressive increase in ceftiofur resistance throughout the studied period was observed. For colistin, a resistance peak (44 % of the strains) was detected in the 2011–2014 period by MIC, which presented a higher sensitivity than the diffusion method. Concerning gentamicin, 10 of 11 strains with intermediate susceptibility by the disk diffusion method were resistant by MIC. The most frequent extended-spectrum beta-lactamases (ESBL) and colistin resistance (*mcr*) genes analysed by PCR were $bla_{\text{CTX-M}}$ (19 % of strains), $bla_{\text{CMY-2}}$ (4 %), *mcr*-4 (15 %), *mcr*-1 (13 %) and in less proportion *mcr*-5 (1 %). Interestingly, these *mcr* genes were already detected in strains isolated in 2000, more than a decade before their first description in 2016. However, poor concordance between the genotypic *mcr* profile and the phenotypical testing was found in this study. These results indicate that although being a current concern, resistance genes and therefore antimicrobial resistant phenotypes were already present in pig farms at the beginning of the century. Special attention should be paid to human last resort drugs like polymyxins and cephalosporins that are still administered to food animals.

Antimicrobial resistance profiles and characterization of *Escherichia coli* strains from cases of neonatal diarrhea in Spanish pig farms.

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E. coli is a common commensal of the gut flora of mammals, and although most strains are not pathogenic, some can cause diarrhea in young pigs. Also, *E. coli* strains can carry antibiotic resistance genes, and therefore, are a good model to monitor antimicrobial resistance.

The aim of this work was to characterize 122 *E. coli* strains isolated from diarrheic animals (n=94) and apparently healthy pen-mates (n=28), and to assess their phenotypical and genotypical resistance patterns.

E. coli strains were analysed for the presence of adhesin (F4, F5, F6, F18, F41 and intimin) and toxin genes (Sta, Stb, VT1, VT2 and EAST1) by PCR. Phenotypical antimicrobial resistance was assessed using the disk diffusion method, and the isolates were further tested for the presence of Extended-Spectrum Beta-Lactamases (ESBL) and colistin resistance genes.

Only 26% of the strains could be classified into a specific pathotype, as the presence of virulence factors genes was, in general, low. More relevant was the high prevalence of multi-resistant *E. coli* strains: 97.5% of the tested isolates showed resistance to 3 or more antimicrobial classes. Statistically significant differences between isolates from diarrheic and healthy animals were found for quinolones and the aminoglycosides gentamycin and neomycin.

Regarding the presence of resistance genes, CTX-M was the only ESBL detected (22%), and a statistically significant relation was found between the presence of this gene and resistance to aminopenicillins, quinolones and aminoglycosides. Colistin resistance genes *mcr*-1 (4/122) and *mcr*-4 (1/122) were also detected.

In conclusion, highly resistant strains harbouring ESBL and colistin resistance genes are present in pig farms, which may pose a threat to animal and human health.

Yellow-legged gulls (*Larus michahellis*) and Audouin's gulls (*Larus audouinii*) from Barcelona as a source of *Campylobacter* of public health relevance

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Wild birds, such as certain gull species, can act as reservoirs of infectious agents and play an important role in the dissemination and maintenance of zoonotic agents, including *Campylobacter*. Yellow-legged gull (*L. michahellis*) populations have increased dramatically, becoming a problem for human health because of its generalist insalubrious scavenging feeding habits, and its increasing direct and indirect interactions with human populations, compared with Audouin's gull (*Larus audouinii*). In Barcelona there is an important urban colony of yellow-legged gull, whilst Audouin's gulls have been reported to breed in the city only once in recent years, in 2013. To gain insight into *Campylobacter* epidemiology in these gull colonies, we assessed the virulence potential (by screening 14 putative virulence genes), the antimicrobial susceptibility (MIC, 6 antimicrobials) and the genetic diversity (by PFGE and MLST) of *C. jejuni* isolates recovered from gull feces in different time periods from 2009-2018. The target virulence genes included those involved in motility, cell adhesion, invasion, cytotoxin production and a gene related to the T6SS. The prevalence of virulence-associated genes was high, indicating that most isolates from both colonies had a high virulence potential. On the contrary, despite the majority of the isolates were pansusceptible, a higher proportion of isolates from yellow-legged gulls, compared with those from Audouin's gulls, showed resistance to antimicrobials of relevance in human medicine. This might be indicative of different feeding habits among this two seagull species and a potential anthropogenic origin of those strains. Also, despite the high genetic diversity among isolates, preliminary MLST analyses show that several *C. jejuni* genotypes (sequence types, STs) have been associated with human gastroenteritis. Overall results highlight the public health risk that these seagull species studied may pose, especially in a human populated area such as Barcelona.

Microbial toxin-based nanoparticles as targeted antitumoral drugs

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Microorganisms have been widely studied as remarkable toxin producers. Pathogenic microorganisms are frequently remembered for their associated detrimental impact causing plenty of diseases such as infections, gastroenteritis, botulism or diphtheria. However, this conception can be changed trying to benefit from their powerful cytotoxicity, for instance in a therapeutic context. Nowadays, conventional cancer treatments are far from being optimal in terms of efficacy. For this reason, the high potency exhibited by some toxins enables toxin-mediated cell killing to be explored in oncology to replace or complement conventional therapies.

In our group, we have genetically engineered microbial toxins like *Corynebacterium diphtheriae* toxin and *Pseudomonas aeruginosa* exotoxin A as potent and selective nanoparticles targeted towards $CXCR4^+$ cancer stem cells. When fused to the ligand T22, toxins specifically bind and internalize $CXCR4^+$ cells, which are present in a variety of human cancers. Once internalized, as they do in nature, both toxins mediate the inactivation of elongation factor 2 (EF-2), which leads to the inhibition of protein synthesis and cell death, whereas CXCR4- cells are not affected by the presence of any T22-empowered toxin. Moreover, when tested in vivo, the systemic administration of both engineered toxins in a colorectal cancer xenograft mouse model promoted efficient and specific local destruction of target tumor tissues and a significant reduction of the tumor volume.

In conclusion, microorganisms and their derivatives (like toxins) have a great therapeutic potential that should be transversally exploited for the treatment of a long list of different human diseases.

Antimicrobial peptide-containing nanoparticles as therapeutics for infectious mastitis treatment

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Mastitis is usually caused by a bacterial infection and is a highly prevalent disease in dairy cattle. It causes economic losses in dairy industry and is responsible for large medication usage, including antibiotics. The emergence and spread of antimicrobial resistance is a relevant problem for animal and human health. Therefore, the development of alternative antimicrobial treatments is urgently needed. Short cationic host defense peptides (HDPs) are secreted by immune and epithelial cells in response to bacterial products and other inflammatory signals. The mechanism of action of HDPs is based on the attachment and disruption of the bacterial membranes. On the other hand, interferon gamma (IFN-γ) is a cytokine that modulates the innate and adaptive immunity against infections. With the aim to study the performance of cationic peptides and the putative synergic action of this type of peptides with IFN-γ, we have recombinantly produced GW-H1, a HDP-inspired cationic peptide, fused to GFP or to interferon gamma (IFN-γ) in addition to a C-terminal His-tag (H6). In previous studies, we have described the ability of modular recombinant proteins designed as cationic peptide-scaffold protein-H6 to induce the formation of soluble protein nanoparticles (NPs). Purified GW-H1-GFP-H6 formed NPs while purified GW-H1-IFN-γ-H6 was not able to self-assemble. We analyzed their performance in the viability of bacteria in cell cultures and in in vivo mastitis animal model. The multiple display of GW-H1 peptide in NPs enhanced the antimicrobial activity of the peptide in *E. coli* cell cultures and in challenged animals while had no activity in the presence of *S. aureus*. However, antibacterial activity was not detected in any of the experiments performed with GW-H1 fused to IFN-γ while the cytokine was active for the infection with *E. coli* in animal model. The results indicate that the biological activity of GW-H1 is promoted in multi display configurations.

Bacterial cellulose matrices to develop enzymatically active paper-based nanocomposites

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The present work focuses on the study of the suitability of bacterial cellulose (BC) matrices to prepare enzymatically active nanocomposites, in a framework of more environmentally friendly methodologies. After BC production and purification, two kind of matrices were obtained: BC in aqueous suspension and BC paper. A lipase was immobilised onto the BC matrices by physical absorption, obtaining Lipase/BC nanocomposites. Neither morphology nor crystallinity, measured by scanning electron microscopy and X-Ray diffractometry respectively, of the CB were affected by the binding of the protein. The activity of Lipase/BC suspension and Lipase/BC paper nanocomposites was tested under different conditions, and the operational properties of the enzyme were evaluated. A shift towards higher temperatures, a broader pH activity range, and slight difference in the substrate preference were observed in the immobilised lipase, compared with the free enzyme. Specific activity was higher for Lipase/BC suspension (4,2 U/mg) than for Lipase/BC paper (1,7 U/mg) nanocomposites. However, Lipase/BC paper nanocomposites showed improved thermal stability, reusability, and durability. Enzyme immobilised onto BC paper retained 60 % of its activity after 48 h at 60 ºC. It maintained 100 % of the original activity after being recycled 10 times and it remained active after being stored for more than a month at room temperature. The results suggested that lipase/BC nanocomposites are promising biomaterials for the development of green biotechnological devices with potential application in bioprocesses and biomedicine. Lipase/BC paper nanocomposite might be a key component of bioactive paper for developing simple, handheld, and disposable devices.

Antimicrobial potential of Host Defense Peptides produced as soluble and nanoclusters-based recombinant proteins

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Antimicrobial resistant bacteria are rising sharply, currently being one of the most critical public health threats. In this context, there is a need to develop alternative antimicrobial molecules and, among them. Host Defense Peptides (HDPs) appear as a promising alternative. HDPs are cationic amphipathic peptides of the innate immunity with a broad-spectrum antimicrobial activity. Although HDPs show desirable therapeutic activities, their production process and short half-life are critical drawbacks. In this scenario, the recombinant production of HDPs has emerged as a promising alternative, since it allows to produce proteins at high production yields. Recombinant technologies also allow to produce the HDP of interest as protein nanoclusters (Inclusion Bodies, IBs), which lead a low-cost product with a sustained release format, reducing treatment doses and avoiding undesirable side effects. Thus, we have focused our study in the development of a novel platform of recombinant HDPs against AMR bacteria, that can be produced in both soluble and protein nanocluster formats. In a first approach, we combined different HDPs in a single polypeptide named JAMF1, and we recombinantly produced it. In a second approach, HDPs such as bovine lingual antimicrobial peptide (LAP), human defensin 5 (HD5) and human defensin 6 (HD6) were produced separately fused to a carrier protein. The analysis of antimicrobial polypeptides and fusion proteins, produced either as IBs or soluble format, showed significant antibacterial activity not only against *Escherichia coli* (up to 97 % of inhibition), but also against multidrug-resistant bacteria such as several strains of *Enterococcus spp.* (up to 94 % of inhibition) and *Klebsiella pneumoniae* quinolone (qnrA) and carbapenem-resistant (91 and 96 % of inhibition, respectively) with an optimal concentration between 0,5 and 3 μM.

Bacterial Cellulose-Chitosan nanocomposites with antimicrobial and antioxidant activity

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The production of paper based bacterial cellulose-chitosan (BC-Ch) nanocomposites was accomplished following two different approaches. In the first, BC paper sheets were produced and then immersed in an aqueous solution of chitosan; in the second, BC paste was impregnated with chitosan prior to the production of the paper sheets. BC-Ch nanocomposites were investigated in terms of some physical characteristics, antimicrobial and antioxidant properties, and ability to inhibit the formation of biofilms on their surface. BC-Ch papers showed a denser network of fibers and smaller pores than BC paper sheets without chitosan. No significant differences in the physical properties were observed between the BC-Ch nanocomposites produced by the two different approaches, maintaining the hydrophilic character, the air barrier properties, and the high crystallinity of the CB paper. Only 5 % of the chitosan leached from the CB-Ch nanocomposites after 96 h of incubation in an aqueous medium, indicating that it was well retained by the BC paper matrix. BC-Ch nanocomposites displayed antimicrobial activity, showing inhibition of growth and killing effect against the bacteria *S.aureus* and *P.aeuruginosa,* and the yeast *C.albicans*. Moreover, BC-Ch papers showed activity against the formation of biofilm on their surface. Paper based BC-Ch nanocomposites combined the physical properties of CB paper and the antimicrobial, antibiofilm and antioxidant activity of chitosan. BC-Ch papers could have applicability in biomedical and packaging fields.

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PÒSTERS

Pòster 1

Study of evolutive adaptation to Tuberculosis in a *Drosophila* model.

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Background:
The progression form latent tuberculosis infection toward an active disease is related to massive neutrophil infiltration of lesions. However, the innate mechanisms capable of inducing the initial neutrophil response are not known. The *Drosophila melanogaster* model has served to understand the innate immune response against multiple infections, due to the high homology of the immune-related genes with humans.

Objectives:
1. To characterize the innate immune response against *Mycobacterium marinum*, which causes a tuberculosis-like disease in flies.
2. To characterize the response triggered by the oral administration of hkMm in the *D. melanogaster* model.

Methods:
Survival of infected flies and bacillary load at different time points have been evaluated to assess the tolerance and resistance profiles considering sexual dimorphism. The response induced by the administration of hkMm (heat-killed *Mycobacterium manresensis*) in *D. melanogaster* subjected to *M. marinum* infection and other stress conditions (High Fat Diet) have also been assessed using the survival and the relative genetic expression of some relevant genes of the immune pathways.

Results:
Tolerance but not resistance varied after *M. marinum* infection depending on sex and the reproductive status. The oral administration of hkMm reduced the mortality related to exposure to High Fat Diet, but did not affect the infection outcome. However, it triggered a systemic immune response immediately after their uptake, while *M. marinum* took up to 5 days to induce an immune response.

Conclusions:
1. There is a sexual dimorphism in tolerance, also influenced by reproductive status.
2. Oral administration of hkMm has an antioxidant effect, reducing mortality of flies exposed to High Fat Diet.
3. *M. marinum* is able to trigger a systemic immune response in the *Drosophila* model at late stages of the infection.
4. Oral administration of hkMm is able to trigger a faster and systemic immune response in the *D. melanogaster* contrary to *M. marinum* systemic infection.

Pòster 2

Modifications in flagella glycosylation affect TLR5 recognition

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Glycosylation is a post-translationally modification found in bacteria, especially in surface proteins, which are modified through the covalent linkage of carbohydrate groups. In particular, *O*-glycosylation of the polar flagellum has been described in several Gram-negative bacteria, where glycans are linked to the hydroxyl group of serine or threonine residues. Mesophilic *Aeromonas* have a single polar flagellum constitutively expressed and involved in adherence and biofilm formation. The filament of this flagellum is constituted by two flagellin subunits (FlaA and FlaB) which are exported when glycosylated and assembled into a helical filament. In *A. piscicola* AH-3, polar flagellins are modified by a heterogeneous heptasaccharide formed by three *N*-acetylhexosamines, two hexoses, and one unknown monosaccharide, linked to the polar flagellins through a pseudaminic acid derivative. The loss of pseudaminic acid derivative abolishes flagellins assembly, and modifications of heteroglycan composition affect polar flagellum stability and motility. Among the different domains comprising the flagellin protein, only D2/D3 is subjected to glycosylation, while D1 has been shown to be highly conserved and recognized by eucaryotic Toll-Like receptor 5. In this work, we determine that modification of the *A. piscicola* AH-3 heptasaccharide decreases flagellin recognition by TLR5. Since the TLR5 recognition sequence on flagellins D1 domain is not directly affected, we suggest that heteroglycan modifications could alter flagellin conformation and therefore, decrease the exposure of D1 domain for TLR5 recognition.

Pòster 3

Production of recombinant mammary serum amyloid A3 and study of its immunomodulatory capability

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Mammary serum amyloid A 3 (M-SAA3) is an acute phase protein secreted locally in the mammary gland. In previous studies, M-SAA3 has presented an important immunomodulatory capability: showing a potential for reducing bacterial translocation into epithelial cells, inhibiting mammary gland infections and stimulating the immune system. However, soluble recombinant M-SAA3 is produced at low yields. In this study we demonstrated that the production of M-SAA3 in *Lactococcus lactis* as inclusion bodies (IBs), also known as protein nanoclusters, is an optimal alternative to obtain pure and soluble protein, through a mild and non-denaturing solubilization, using N-lauroylsarcosine detergent. Moreover, the activity of M-SAA3 at stimulating the innate immune system was assessed *in vitro* using soluble protein or directly the M-SAA3 IBs. Interestingly, the increase in IL-8 expression, which is one of the main cytokines modulated by M-SAA3, was greater with the M-SAA3 IBs than with soluble M-SAA3 when low concentrations (10 ug/mL) were used. Nevertheless, at higher concentrations of protein (75 ug/ml), the soluble format was more effective than protein nanoclusters. In order to study if this result was a consequence of the slow release existing in the IBs, a pre-incubation of M-SAA3 IBs was done in PBS, during 16, 24 and 48 h. The recovery of the supernatant by centrifugation of preincubated IBs lead a pool of active molecules able to activate the expression of IL-8 at higher levels than initial IBs material from which derived. To verify this immune stimulation, expression of IL-6, IL-10 and IFNγ was analyzed, observing similar profiles than IL-8 . In conclusion, this study demonstrated that M-SAA3 IBs obtained in bacterial expression systems can be a source of soluble and pure protein and a new active format, whose action is based on the slow-release of soluble and active molecules.

Pòster 4

Safety and toxicity study of the immunomodulatory agent *Mycobacterium brumae* in two different animal models

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Intravesical BCG immunotherapy represents the standard gold treatment for non-muscle-invasive bladder cancer patients, although half of the patients develop side effects related to this therapy. Exploring BCG-alternative therapies, the non-tuberculous *Mycolicibacterium brumae* (basonym *Mycobacterium brumae*), has shown outstanding antitumoral and immunomodulatory capabilities. Although none infection due to *M. brumae* in humans, animals, or plants has been described, the safety and/or toxicity of this mycobacterium has not been previously addressed.
In the present study the progression of *M. brumae* and BCG in intravenous-infected SCID mice and infected *Galleria mellonella* larvae were studied over 105 or 6 days, respectively. At the end of the study, the hemolymph from larvae and spleen, liver and lung from infected mice were processed for CFU counts. Blood samples from mice were taken and a broad range of hematological and biochemical parameters were analyzed. Finally, histopathological signs in mice tissues were studied.
While 100% and 90% of *M. brumae*-infected mice and larvae, respectively, survive until the end of the study, BCG-infected mice survived over 48 days and BCG-infected larvae survival dropped to 42%. *M. brumae* cells were not found in any mice or larvae biological sample, whereas BCG counts were found in all processed tissues from both animal models. Contrary to those found in BCG-infected mice, none hematological, biochemical and histopathological signs of infection were found in *M. brumae*-infected mice.

To conclude, *M. brumae* has turned out to be a safe therapeutic biological agent overcoming the security and toxicity disadvantages presented by BCG in both mice and *Galleria mellonela* animal models.

Pòster 5

Novel Oleanolic and Maslinic Acids derivatives as a promising treatment against bacterial biofilm in nosocomial infections: An i*n vitro* and *in vivo* study

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Oleanolic Acid (OA) and Maslinic Acid (MA) are pentacyclic triterpenic compounds that abound in the industrial olive-oil waste. These compounds have renowned antimicrobial properties and lack cytotoxicity in eukaryotic cells as well as resistance mechanisms in bacteria. Despite these advantages, their antimicrobial activity has only been tested *in vitro*, and derivatives improving this activity have not been reported. In this work, a set of 14 OA and MA C-28 amide derivatives have been synthesized. Two of these derivatives, MA-HDA and OA-HDA, increase the *in vitro* antimicrobial activity of the parent compounds while reducing their toxicity in most of the Gram-positive bacteria tested, including a methicillin-resistant *Staphylococcus aureus*-MRSA. MA-HDA also shows an enhanced *in vivo* efficacy in a *Galleria mellonella* invertebrate animal model of infection. A preliminary attempt to elucidate their mechanism of action revealed that these compounds are able to penetrate and damage the bacterial cell membrane. More significantly, their capacity to reduce antibiofilm formation in catheters has also been demonstrated in two sets of conditions: a static and a more challenged continuous-flow *S. aureus* biofilm.

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Pòster 6

Comparative transcriptomics to identify gene expression differences between AIEC and non-AIEC isolates during *in vitro* cell infection

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Adherent-invasive *Escherichia coli* (AIEC) have been involved in the etiology of Crohn's disease. Current techniques for AIEC identification, based on phenotypic assays on infected cell cultures, are non-standardisable and time-consuming. Previous studies related to AIEC genome, showed the absence of specific sequences or mutations widely distributed across all AIEC strains. Therefore, no molecular signatures are available to identify AIEC phenotype. We aimed to detect differentially expressed genes between AIEC and non-AIEC clinical isolates in order to determine if differences in the expression of particular genes are responsible of the AIEC phenotype.

Two AIEC/non-AIEC strain pairs displaying different phenotype but identical pulsotype, and each pair belonging to a different phylogroup (B1 and D), were analysed during I-407 cells infection. Two conditions were considered: the supernatant fraction of the infected cell cultures (SN) and the fraction adhered and/or invading the intestinal epithelial cells (A/I). Comparative transcriptomics were performed and differences validated by RT-qPCR using Fluidigm. Moreover, differentially expressed genes were further studied in a collection of 14 AIEC and 26 non-AIEC strains.

Sixty-seven differentially expressed genes were found between the two phenotypes, including 19 over-expressed and 48 under-expressed genes in AIEC strains. So far, 49 genes were subsequently validated with Fluidigm, of which 33 (67.35%) were corroborated. Functional analysis suggested that the most abundant category was that including genes related with metabolic processes (10/33), followed by those with unknown function (9/33).

No previous comparative transcriptomic studies have been performed using AIEC-infected cell cultures. Differentially expressed genes have been detected, and they may be involved in AIEC pathogenicity. However, further studies are needed to determine if the differential gene expression is widely among AIEC strains and to assess the role of these genes in the AIEC phenotype.

Pòster 7

Ultrastructural external and internal analysis of *Mycobacterium brumae* grown on differentculture media

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Lipid bodies and outer cell wall lipids are complex and abundant in mycobacteria, even so, their potential relevance is under discussion. The lipid bodies abundance and type varies depending on culture medium, the growing conditions and the age of the culture. We hypothesize that in *Mycolicibacterium brumae* (basonym *Mycobacterium brumae*), a non-pathogenic mycobacterium that have antitumor efficacy on nonmuscle-invasive bladder cancer, the differences in external and internal lipids patterns observed when is grown in different Sautons, could be related with different degrees of tumor inhibition, being a factor to take into account in the development of future treatments. In the present study, the lipid bodies and the outer apolar cell wall lipids of *M. brumae* grown of different media are analyzed by using Thin Layer Chromatography (TLC) and Scanning, Field Emission and Transmission Electron Microscopy (SEM, FE-SEM and TEM).

Pòster 8

Genetic diversity and virulence potential of *Campylobacter coli* and *Campylobacter lanienae* carried by wild boars from the metropolitan area of Barcelona

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The contribution of wildlife to human campylobacteriosis is still largely unknown. This study aimed to gain insight into the epidemiology and virulence potential of *C. coli* and *C. lanienae* isolated from wild boars from the metropolitan area of Barcelona (NE Spain), where this species has colonized urban and peri-urban areas. The recovered isolates of 78 *Campylobacter*-positive wild boars, out of 133 sampled, were initially typed by pulsed field gel electrophoresis (PFGE). Representatives of the different pulsotypes were analyzed by multilocus sequence typing (MLST) and screened by PCR for the presence of 14 putative virulence genes. A high genetic diversity was observed amongst both *Campylobacter* species with 12 different sequence types (STs), of which 4 were novel among *C. coli* isolates; ST-854 was the most prevalent. All *C. lanienae* isolates belonged to novel STs. In both *Campylobacter* species some genotypes (ST-854, ST-827, ST-9235 in *C. coli*) were found in the different studied areas (urban, semi-urban and natural areas). Besides the novel STs, all other *C. coli* STs have been associated with human gastroenteritis. Some virulence genes like *flaB*, *ceuE* and *hcp* were detected in the majority of the isolates, whereas the prevalence of other genes (*ciaB, racR* and *wlaN*) was low. Four isolates were positive to up to 12 of the 14 virulence genes analyzed, suggesting a high pathogenic potential. Overall results show that wild boars in the metropolitan area of Barcelona are carriers of *Campylobacter* genotypes which may pose a public health threat due to their increasing contact with humans.

Pòster 9

Evaluation of the immune response in tuberculosis patients before and after receiving therapeutical surgery in Georgia

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Introduction: In Georgia, a country which faces a high tuberculosis (TB) burden, therapeutic surgery is routinely practiced on TB patients to avoid the spread of the disease and cope with sequels, following WHO criteria. The aim of this work was to evaluate the immune responses of a cohort of 40 Georgia TB patients that underwent therapeutic surgery.

Objectives: 1) to measure immune biomarkers levels before and after therapeutical surgery and (2) to analyze the results according to clinical, epidemiological severity factors.

Methods: Clinical and epidemiological factors were recorded for each patient. ELISA and Luminex immune-assays were performed to measure cytokines, chemokines and innate response proteins from plasma and urine samples, collected before and after surgery. The results were analyzed according to several disease severity factors.

Results: Differences in biomarkers levels were found due to sex and toxic habits; inflammatory markers correlated with the presence of cavities, radiological characteristics and presence of bacilli in tissue; levels of most of biomarkers were increased after 11 days post-surgery.

Conclusions: Correlation was found between systemic biomarkers and several epidemiological and clinical factors. A signature combining epidemiological factors and biomarkers could be used to predict the persistent presence of bacilli in TB lesions in spite of treatment completion.

Pòster 10

Differences in the tumor proliferation inhibitory ability among strains of the same *mycobacterium* species

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The use of *Mycobacterium bovis* Calmette-Guerin bacillus (BCG) as local immunological therapy for bladder cancer treatment remains nowadays the most successful immunotherapeutic tool due to its high effectiveness. However, serious adverse events including cases of BCG infection in treated patients are described. *Mycolicibacterium brumae* (basonym *Mycobacterium brumae*) was described in 1993 as an environmental mycobacterium and none cases of infection has been reported in humans or animals. Previous research in our group has demonstrated that the type strain of *M. brumae* shows a direct antitumor capacity on different both human and murine bladder cancer cells (T24, 5637, SW780 and MB49); demonstrating a similar or superior tumor cell inhibition to BCG treatment. Furthermore, *M. brumae* trigger an immune response inducing an adequate interleukin release, both *in vitro* and *in vivo*, showing to be a good candidate to replace BCG in BC treatment. None is known about the antitumoral and immunotherapeutic capacity of other *M. brumae* strains than the type strain. Here, we made an *in vitro* phenotypic and antitumor activity analysis of four *M. brumae* strains against bladder, lung, colon, pancreas, cervix and breast cancer cell lines with different histopathology grade.

Pòster 11

Estudi i avaluació de nous probiòtics

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Els probiòtics són microorganismes vius que a l'administrar-se en quantitats adequades confereixen un benefici per a la salut de l'hoste. Degut als nombrosos beneficis que aporten, en els últims anys, la seva investigació ha experimentat un gran auge obtenint diversos avenços científics que han conclòs amb la comercialització de diferents productes.

Hi ha diferents gèneres bacterians que poden tenir un potencial probiòtic essent un exemple el gènere *Lactobacillus.* En el grup de recerca es treballa principalment amb *Lactobacillus plantarum* CA-7. Aquest bacteri va ser obtingut en estudis previs amb cargols i s'ha demostrat el seu potencial probiòtic.

Aquests bacteris per poder ser consumits i arribant en una quantitat adequada en l'intestí i així poder realitzar el seu efecte s'han de preservar. Una de les tècniques més emprades per aconseguir-ho és la liofilització.

La liofilització és un mètode de dessecació mitjançant un procés de congelació i una posterior sublimació. Per protegir els bacteris d'aquests processos s'afegeix un crioprotector.

En aquest moment, la nostra recerca es centra en l'avaluació de diferents crioprotectors i combinacions d'aquests per avaluar quin és el que proporciona una major supervivència d'aquest bacteri en concret.

Finalment, aquest liòfil s'afegeix en pinso de cargols per avaluar el seu efecte en la microbiota intestinal d'aquest animal.

Pòster 12

Dynamics of *Campylobacter* infection in a colony of yellow-legged gulls (*L. michahellis*)

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The dramatic increase in the populations of the yellow-legged gull (*Larus michahellis*) has become a problem for human health because of its insalubrious scavenging feeding habits, and its increasing direct and indirect interactions with human populations. In this regard, we evaluated the infection dynamics of the zoonotic bacteria *Campylobacter* spp. among adults and their offspring within a gull colony in Ebro Delta as well as the virulence potential of their isolates. We sampled cloacal swabs from 17 adult gulls, as well as their offspring at two different periods (3-5 day-old and 3 week-old), for *Campylobacter* spp. isolation. Isolates were genotyped by pulsed-field gel electrophoresis and multi-locus sequence typing (MLST), and were also PCR-screened for the presence of 14 putative virulence factors. We isolated *Campylobacter jejuni* (6 positive chicks), *C. lari* (1 adult, 3 chicks) and *C. coli* (1 chick). We detected 7 MLST sequence types (STs), 4 (57%) of which were novel to the PubMLST database (3 from *C. lari,* 1 from *C. jejuni*). A high genetic diversity was found, with the same genotype detected mainly only among chicks of same age in some nests, but different from that of the positive adult. Nestlings may get infected by *Campylobacter* from the environment and transmit the infection to siblings of the same nest, and to other chicks in the colony. The prevalence of virulence-associated genes was high (71%), thus pointing to a high virulence potential of most isolates.

Pòster 13

Biodistribution and transcytosis of liposome-encapsulated bacteriophages in oral phage therapy

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The present work studies the biodistribution of orally administered, liposome-encapsulated bacteriophages and their transcytosis through intestinal cell layers. The use of fluorochrome-labeled bacteriophages together with a non-invasive imaging methodology allowed the *in vivo* visualization of bacteriophages in the stomach and intestinal tract of mice. In these studies, phage encapsulation resulted in a significant increase of phages in the mouse stomach, even 6 h after phage administration. By contrast, encapsulated and non-encapsulated phages were equally visualized in the intestine. These *in vivo* observations were corroborated by culture methods and *ex vivo* experiments. The percentage of encapsulated phages in the stomach remained constant (50%) up to 6 h post-administration. The *ex vivo* experiments also revealed a higher concentration of non-encapsulated than encapsulated phages in liver, kidney, and even muscle up to 6 h post-administration. Moreover, encapsulated bacteriophages were able to reach the liver, spleen, and muscle. The application of confocal laser scanning microscopy in *in vitro* cultures of human intestinal cells (Caco-2/HT29/Raji-B) revealed that Vybrant-Dil-stained liposomes containing labeled bacteriophages were preferably embedded in cell membranes. Although no transcytosis of encapsulated phages was detected in this *in vitro* model, SYBR-gold-labeled non-encapsulated bacteriophages were visualized crossing the membrane.

Our work evidenced the prolonged persistence of the liposome-encapsulated phages in the stomach and their adherence to the intestinal membrane. We propose a model where the liposome-encapsulated phages could suffer a process of transcytosis reaching several internal organs in their encapsulated form or released from their capsules. These observations could explain the greater long-term efficacy of phage therapy using liposome-encapsulated phages.

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Pòster 14

Mechanism of action of NrdR, a system confined to bacteria behind the control of all dNTP synthesis

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Any living cell requires DNA replication and repair. The building blocks for these activities are the deoxyribonucleotides (dNTPs), which are formed by the action of a single, complex family of enzymes, the ribonucleotide reductases (RNR).

As dNTP synthesis is essential, RNRs have been proposed as promising targets for antimicrobial therapies, and have already been successfully used for cancer treatment; any drug able to suppress or severely affect RNR activity will undoubtedly kill the target cell. However, we need to understand the mechanism of action and regulation of the RNRs to design effective and specific drugs.

The regulation of bacterial RNR activity is the main focus of this study. Although each specific RNR is regulated by different mechanisms in each bacterial species, there is a single system that can control all RNR activity in bacteria while being completely absent in eukaryotic cells: the transcription factor NrdR. The NrdR protein was first discovered in *Streptomyces coelicolor*, but we now know that it appears as a global RNR repressor in almost all bacterial species. Its mechanism is mostly unknown, although it has been proposed that it binds nucleotides and represses all RNR classes when enough dNTPs are present. The incapacity to obtain pure and stable NrdR has severely limited all research in the field.

Here we present a project in collaboration with the Structural Biology Unit of the Molecular Biology Institute of Barcelona (SBU, IBMB-CSIC) aimed toward fully understanding the mechanism of action of NrdR. We describe a method to obtain good yields of pure and stable NrdR protein and saturate it with any nucleotide cofactor. We use size exclusion chromatography and Multi-Angle Light Scattering (MALS) to demonstrate that NrdR responds to the nucleotide bound by forming different oligomeric structures. We then use Electrophoretic Mobility Shift Assays (EMSAs) and *in vitro* transcription techniques to determine the physiological meaning of each of the oligomeric forms. These results are combined with general transcriptomics by RNA-seq, *in vivo* studies and bioinformatical techniques to draw the first model of the NrdR regulation mechanism.

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Pòster 15

Bottom-Up Instructive Quality Control in the Biofabrication of Smart Protein Materials

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Since the approval of insulin in 1981, ≈400 protein drugs, mainly produced in microbial cells, have been authorized for use in humans. Apart from plain therapeutic cytokines, hormones, enzymes, and antibodies, a plethora of more elaborate protein structures with varying complexity have been developed as nano-conjugates for drug delivery.

The impact of cell factory quality control on material properties is a neglected but critical issue in the fabrication of protein biomaterials, which are unique in merging structure and function. We have analyzed the influence of bacterial quality control on hierarchical structural features and the biological performance of smart protein materials of biomedical interest, illustrated by T22GFPH6, a tumor-targeted, self-assembling nanoparticle produced by recombinant methods. This protein spontaneously self-assembles into nanoparticles of ≈15 nm and when systemically administered in colorectal cancer mice models, these particles escape renal filtration and target primary tumor and metastatic foci, through specific internalization in CXCR4 + cells. To evaluate to which extent the protein production/folding machinery might impact protein self-assembly, and thus influence architectonic features and function of T22-GFP-H6 nanoparticles, the building block was produced in E. coli K-12 strains with knocked-out agents, critical in different arms of the protein quality control.

We concluded that the molecular chaperoning of protein conformational status is a potent molecular instructor of the macroscopic properties of self-assembling, cell-targeted protein nanoparticles, including biodistribution upon in vivo administration.

Pòster 16

High Time Resolution and High Signal-to-Noise Monitoring of the Bacterial Growth Kinetics in the Presence of Plasmonic Nanoparticles

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Background. Emerging concepts for designing innovative drugs (i.e., novel generations of antimicrobials) frequently include nanostructures, new materials, and nanoparticles (NPs). Along with numerous advantages, NPs bring limitations, partly because they can limit the analytical techniques used for their biological and *in vivo* validation. From that standpoint, designing innovative drug delivery systems requires advancements in the methods used for their testing and investigations. Considering the well-known ability of resazurin-based methods for rapid detection of bacterial metabolisms with very high sensitivity, in this work we report a novel optimization for tracking bacterial growth kinetics in the presence of NPs with specific characteristics, such as specific optical properties.

Results. Arginine-functionalized gold composite (HAp/Au/arginine) NPs, used as the NP model for validation of the method, possess plasmonic properties and are characterized by intensive absorption in the UV/vis region with a surface plasmon resonance maximum at 540 nm. Due to the specific optical properties, the NP absorption intensively interferes with the light absorption measured during the evaluation of bacterial growth (optical density; OD_{600}). The results confirm substantial nonspecific interference by NPs in the signal detected during a regular turbidity study used for tracking bacterial growth. Instead, during application of a resazurin-based method (Presto Blue), when a combination of absorption and fluorescence detection is applied, a substantial increase in the signal-to-noise ratio is obtained that leads to the improvement of the accuracy of the measurements as verified in three bacterial strains tested with different growth rates (*E. coli*, *P. aeruginosa,* and *S. aureus*).

Conclusions. Here, we described a novel procedure that enables the kinetics of bacterial growth in the presence of NPs to be followed with high time resolution, high sensitivity, and without sampling during the kinetic study. We showed the applicability of the Presto Blue method for the case of HAp/Au/arginine NPs, which can be extended to various types of metallic NPs with similar characteristics. The method is a very easy, economical, and reliable option for testing NPs designed as novel antimicrobials

Pòster 17

Prevalence of carbapenem and CR genes in stool samples of wild boars of Barcelona region

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The spread of antimicrobial resistant *Enterobacteriaceae* within the environment is a global concern. Wildlife such as wild boars have been described as a possible reservoir of antimicrobial resistant bacteria. In Barcelona, as in many other European cities, there are populations of wild boars living very close to the urban nucleus, representing a possible focus of maintenance and development of resistant *Enterobacteriaceae* population. In the present study we focused to determine the prevalence of different resistance genes in stool samples from wild boar of Barcelona County. Extended-spectrum β-lactamases (ESBLs) and AmpC-type β-lactamases (AmpC) are the most common enzymes that confer resistance to broad-spectrum cephalosporins among members of the *Enterobacteriaceae* family. In this study, 68 wild boars were sampled from November 2017 to April 2018. Stool samples were cultured on MacConkey agar, and *Escherichia coli* isolates DNA were extracted and analyzed by PCR for common carbapenem and cephalosporine resistant (CR) genes: ESBLs (blaCTX-M, blaTEM, blaSHV), AmpCs (blaCMY-1, blaCMY-2) and carbapenemases (blaOXA, blaCLR and blaVIM). *E.coli* was identified in 59/68 animals. Overall prevalence of CR-phenotype was 27% (16/59), distributed as follow: 10.2% (6/59) blaTEM, 8.5% (5/59) blaCMY-2, 3.4% (2/59) for both blaCTX-M and blaSHV and 1.7% (1/59) blaCMY-1. No resistant genes for carbapenems were found. This study confirms that wild boars living in close contact with urban areas can carry CR-resistance genes, representing a potential risk for the transmission of these antimicrobial resistance in the wild and for the Public Health.