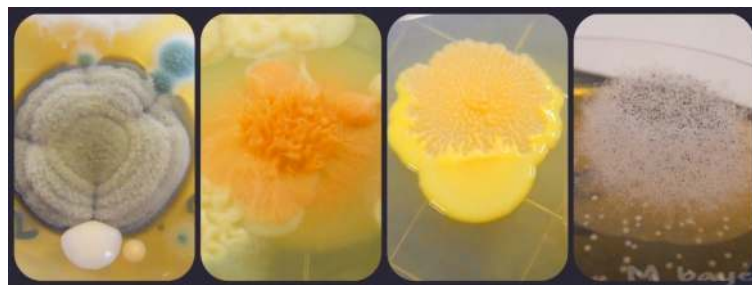


I Jornada de Microbiologia



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



INSTITUT D'ESTUDIS CATALANS

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Societat Catalana
de **BIOLOGIA**

I Jornada de Microbiologia

***Organitzada per la Secció de Microbiologia de la
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PROGRAMA

Comitè Organitzador:

Montserrat Bayés
Microbiology Quality Management

Eduard Torrents Serra
Group Leader Institut de Bioenginyeria de Catalunya (IBEC)

Jordi Mas
Professor Associat UAB, UIC

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Dimarts, 26 d'abril de 2016

Sessió MATÍ

09:00 - 09:30 **Recollida de documentació**

Moderador: Eduard Torrent, Institute for Bioengineering of Catalonia (IBEC)

09:30 - 10:00 **CONFERÈNCIA INAUGURAL**

Antoni Villaverde

Microbial fabrication of nanostructured protein materials
IBB, Dept. de Genètica i de Microbiologia, UAB

10:00 - 10:30

The potential of molecular techniques for the management of pathogens in the meat industry
Teresa Aymerich, Belen Martín, Margarita Garriga, IRTA

10:30 - 11:00

Taxonomy of bacteria in the genomic era
Maria Jose Figueras, Dept. Ciències Mèdiques Bàsiques, URV

11:00 - 12:00 **Sessió de pòsters – Coffee break**

12:00 - 12:15

Genetic and phenotypic diversity of the opportunistic pathogen Malassezia pachydermatis
Laura Puig, G. Castellá, M.R. Bragulat, F.J. Cabañes, Veterinary Mycology Group, UAB

12:15 - 12:30

Computational methods to explore viral species from wastewater metagenomics data
Natalia Timoneda et al., Laboratori de Virus Contaminants d'Aigua i Aliments (VIRCONT), Dept. de Genètica, Microbiologia i Estadística, Secció de Microbiologia, Virologia i Biotecnologia, UB

12:30 - 12:45

Polyphosphate is involved in cell cycle progression and genomic stability in Saccharomyces cerevisiae
Samuel Bru et al., Faculty of Medicine and Health Sciences, UIC

12:45 - 13:00

Water treatments for hepatitis E virus: UV radiation and flocculation-chlorination sachets
Laura Guerrero-Latorre et al., Laboratori de Virus Contaminants d'Aigua i Aliments (VIRCONT), Dept. de Genètica, Microbiologia i Estadística, Secció de Microbiologia, Virologia i Biotecnologia, UB

13:00 - 13:15

A method for monitoring bacterial gene expression as determined by oxygen concentration
Lucas Pedraz et al., Bacterial infections and antimicrobial therapies group, IBEC

13:15 - 13:30

Virulence factors on Mucor circinelloides strains: a comparative study
Loida López, Dept. Ciències Mèdiques Bàsiques, URV

13:30 - 15:30 **Dinar**

Sessió TARDA

Moderador: Jordi Mas, UAB, UIC

15:30 - 15:45

Predictive microbiology as an emerging approach for assessing microbial inactivation by high pressure processing

Sara Bover-Cid, Anna Jofré, Cristina Serra, Margarita Garriga, IRTA

15:45 - 16:00

Fagoteràpia i resistència als bacteriòfags.

Jennifer Otero, Grup de Microbiologia Molecular, UAB

16:00 - 16:15

Application of ERIC-PCR genotyping method for screening the prevalence of three cryptic species uncover by Aeromonas media clinical and environmental strains

Ana Fernández Bravo, Dept. Ciències Mèdiques Bàsiques, URV

16:15 - 16:30

A continuous microalgal photobioreactor for emerging contaminants removal in urban wastewater: set up and community study

Eloi Parladé et al. Dept. de Genètica i de Microbiologia, Escola d'Enginyeria, UAB; Institut Català de Recerca de l'Aigua, ICRA

16:30 - 17:00 **Pausa**

17:00 - 17:15

Culture media with NaCl enhance the recovery of Arcobacter spp. from water and shellfish. Re-assesment of the traits of the rare species Arcobacter marinus and Arcobacter halophilus

Nuria Salas Massó, Dept. Ciències Mèdiques Bàsiques, URV

17:15 - 17:30

Com esquiven les poblacions bacterianes els compostos genotòxics?

Oihane Irazoki, Grup de Microbiologia Molecular, UAB

17:30 - 17:45

Next generation sequencing as a tool to diagnose new potential viral causes in non-A-E hepatitis patients

Eloy A Gonzales Gustavson, Laboratori de Virus Contaminants d'Aigua i Aliments (VIRCONT), Dept. de Genètica, Microbiologia i Estadística, Secció de Microbiologia, Virologia i Biotecnologia, UB

17:45 - 18:00

The field of microbiology in research projects of the Horizon 2020 Framework EU Program

Jordi Mas, Secció de Microbiologia, Societat Catalana Biologia, IEC

18:15 - 18:30 **Cloenda**

PÒSTERS

1. *In vivo efficacy of antifungal combination therapies against Aspergillus fumigatus.* **Adela Martin**, Dept. Ciències Mèdiques Bàsiques, URV.
2. *In vitro data are predictive of the in vivo efficacy of voriconazole in murine infection by Candida glabrata.* **Marta Sanchis**, Dept. Ciències Mèdiques Bàsiques, URV.
3. *MALDI-TOF and rpoB sequencing reveals previously undetected cases of Arcobacter butzleri from human diarrhoea.* **Alba Pérez-Cataluña**, Dept. Ciències Mèdiques Bàsiques, URV.
4. *Microbiological and enzymatic characterization of pregnant mare's milk. Evaluation of its probiotic, antimicrobial and preservative properties.* **Gisela Girmé Vila**. Grup de Recerca en Microbiologia Aplicada i Medi Ambiental, UAB.
5. *Microbiological quality of olives and antimicrobial action.* **Graciela C. Santos**¹, E. L. Arosema, J. M. Planas², M. A. Calvo Torras¹, ¹Dept. Health and Anatomy Animals, UAB, ²Dept. Physiology, UB.
6. *Determinació de compostos orgànics volàtils de cultius fúngics mitjançant SPME-GC-MS.* **Lluís Pons Anglada**, Joan Josep Gallardo Chacón, Maria dels Àngels Calvo Torras, Dept. Sanitat i Anatomia Animals, Fac. Veterinària, UAB.
7. *Microbiología del caracol Helix aspersa Müller, y aplicaciones biotecnológicas para su mejoramiento sanitario con impacto en su comercialización.* **Rodrigo Vera, Esteban Arosema, M. A. Calvo**, Grup de Recerca en Microbiologia Ambiental i Aplicada, Fac. Veterinària, UAB.
8. *Deciphering the involvement of ribonucleotide reductase in adherent-invasive Escherichia coli LF82 infection.* **Nicolas Dreux, Maria del Mar Cendra, Arlette Darfeuille-Michaud, Nicolas Barnich**. Bacterial infections and antimicrobial therapies group. Institute for Bioengineering of Catalonia (IBEC).
9. *Pseudomonas aeruginosa exhibits deficient biofilm formation in the absence of class II and III ribonucleotide reductases due to hindered anaerobic growth.* **Anna Crespo, Lucas Pedraz, Josep Astola and Eduard Torrents**. Bacterial Infections and Antimicrobial Therapies. Institut de Bioenginyeria de Catalunya (IBEC).
10. *Improving wound healing through infection treatment.* **Aida Baelo, Alba Pérez and Eduard Torrents**. Bacterial Infections and Antimicrobial Therapies. Institut de Bioenginyeria de Catalunya (IBEC).
11. *Antimicrobial effect of atmospheric DBD plasma onto packaged solid samples with different atmospheres.* **Sara Peiró, María Parra, Edurne Gastón**,

Alejandro Rosales, Laura Rodríguez. IRIS-Innovació i Recerca Industrial i Sostenible. Parc Mediterrani de la Tecnologia.

12. *Microorganismes i patrimoni cultural.* **Esteban Leonardo Arosemena Angulo.** Grup de Recerca en Microbiologia Aplicada i Medi Ambiental, UAB.
13. *Microbiota de leche de yegua: efectos beneficiosos para la salud humana y animal.* **Brian Morales Segovia.** Grup de Recerca en Microbiologia Aplicada i Medi Ambiental, UAB.
14. *American Society for Microbiology. Country Ambassador Program.* **Maria Jose Figueras,** Dept. Ciències Mèdiques Bàsiques, URV.



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COMUNICACIONS

Microbial fabrication of nanostructured protein materials

Ugutx Unzueta^{1,2,3‡}, Fabián Rueda^{1,2,3}, Olivia Cano-Garrido^{1,2,3}, Mireia Pesarrodonà^{1,2,3}, Naroa Serna^{1,2,3}, Laura Sánchez-García^{1,2,3}, María Virtudes Céspedes^{3,4}, Joaquín Seras-Franzoso^{1,2,3}[†], Rafael Cubarsi⁵, Alejandro Sanchez-Chardi⁶, Mónica Roldán⁶, José Luis Corchero^{1,2,3}, Oscar Conchillo-Solé¹, Xavier Daura⁷, Ramón Mangues^{3,4}, Neus Ferrer-Miralles^{1,2,3}, Esther Vazquez^{1,2,3}, Elena García-Fruitós^{1,2,3}[†], Antonio Villaverde^{1,2,3}.

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Microbial cells produce a remarkable spectrum of nanostructured materials of biotechnological and biomedical interest. In particular, self-assembling proteins, obtained by recombinant technologies, offer wide potentialities and clear advantages over other categories of nanomaterials explored in medicine. This is because of their biocompatibility, moderate complexity of fabrication, low production costs, full scalability and functional and structural versatility that can be regulated by conventional genetic engineering. We have explored how features of the microbial cell factory, such as the selected producer species or a given genetic background, might influence relevant properties of protein materials. For that, we have selected as models two categories of protein assemblies with emerging applications in biomedicine, namely functional, drug-releasing nanostructured amyloids and tumor-targeted protein nanoparticles. Interestingly, we have recognized a profound impact of the cell's quality control on materials' properties at the macroscopic level. This fact reveals a strong dependence of the functionalities of complex protein materials on the cellular protein folding machinery in a hierarchical, bottom-up fashion.

The potential of molecular techniques for the management of pathogens in the meat industry

Teresa Aymerich, Belen Martín i Margarita Garriga

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The application of an integrated approach to food safety has provided Europe with one of the safest food supplies in the world, contributing to food and nutrition security and also supporting economy and trade. Nevertheless, recent disease cases and outbreaks, as reported by EFSA (2015), have revealed that food-borne microbial diseases are still a matter of concern.

To produce safe food, collaboration between the food, beverage and agricultural industries within a farm-to-table approach, together with the application of the preventive measures based on HACCP plans and scientific risk assessment is considered a must. Effective verification of the correct application of the plans, traced along the food chain, together with the assurance of the accomplishment of the microbiological criteria is considered essential. In this way, rapid detection and identification of microbial agents, enabling timely response and facilitating early detection of faults and deviations plays a crucial role to accelerate decision making in production, to control spread and food safety but also to avoid recalls and keep the enterprise image and consumers' confidence.

The rapid development of genomic technologies uphold a great promise for improving specific early detection, prevention and control of current and emerging food-borne microbial pathogens thus contributing to improved health of consumers. Indeed rapid detection methods for food-borne pathogens and spoilage microorganisms are being adopted by food industry and the number of commercially validated molecular available kits and methods for rapid detection of food-borne pathogens continues to increase. Inclusivity, specificity, reproducibility, sensitivity, viability together with speed, throughput, flexibility and cost will be considered to assess the criteria of how to use a specific method for a specific target.

Keywords: Food safety, Food-borne pathogens, Molecular techniques, Meat industry.

Taxonomy of bacteria in the genomic era

Maria Jose Figueras

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The taxonomy of bacteria is based on common requirements for assigning a strain or a group of strains to a new species and includes, both a phenotypic and a molecular characterization. From the phenotypic point of view at least one distinctive phenotypic characteristic to differentiate the new species from existing species is required, and molecular characterization rely on the comparison of 16S rRNA gene sequences (> 1300bp) and DNA–DNA hybridization (DDH) results with the closely related species (classically those with 16S rRNA similarities >97% or >98.5-99 %). The cut off value established for delimiting species on the basis of the DDH, have been set at 70-80%. More recently, the Multilocus Sequence Analysis (MLSA) or the Multilocus Phylogenetic Analysis (MLPA) using of at least five concatenated housekeeping genes is considered an accurate tool for the delineation of bacteria species because it shows a higher resolution than the 16S rRNA gene. Therefore its use has been proposed as an alternative for DNA hybridization. New sequencing technologies enabled to obtaining the genome sequence of a microorganism easier and at a relatively low cost, and so the number of available bacteria genomes had increased exponentially in recent years. This had led to the development of new tools for defining bacteria species like the average nucleotide identity (ANI) that compare the homologous genes shared between two genomes, ANI values >95-96% showed to correlate with DDH results >70%. So the use of ANI has been proposal as the "gold standard" for species delineation using genomes. Using this tool we have discovered that 36% of the available *Aeromonas* genomes deposited at the GenBank database were mislabelled. Considering this finding we recommended to verify the correct identity of a genome sequence using ANI or doing a MLPA before a genome is submitted to the database. Our experience on the description of new species of the genus *Arcobacter* and *Aeromonas* will be presented giving our view on the strategy for discovery new species and presenting the approach used by other authors.

Genetic and phenotypic diversity of the opportunistic pathogen *Malassezia pachydermatis*

L. Puig, G. Castellá, M.R. Bragulat, F.J. Cabañes

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Presenter: laura.puig@uab.es

Malassezia pachydermatis is part of the normal microbiota of carnivores, although under certain conditions it can cause dermatitis and otitis, especially in dogs and cats. A great variability exists among isolates of this species, which has been studied principally with molecular methods using isolates from dogs. The aim of this work was to study the phenotypic and molecular diversity of *M. pachydermatis*.

Isolates from various animal species and different health status were studied with a multilocus phylogenetic approach, sequencing ITS rRNA, LSU rRNA, CHS-2 and β -tubulin genes. Different sequence types were identified for each gene, some of which were described for the first time. Combining the sequences of the four loci in the multilocus phylogenetic analysis, fifteen genotypes were identified, distributed in two clades. The first clade contained genotypes from cats, dogs, a goat and a pig, while the second clade included genotypes from dogs and one from a horse. A clear relationship was observed between *M. pachydermatis* genotypes and specific animal species, regardless of the health status of the animal.

Phenotypic features of the strains were also characterized. Growth was evaluated at different incubation temperatures and the catalase and β -glucosidase activity were assessed. Assimilation of different polysorbates (tween 20, 40, 60, 80) and Cremophor EL was also evaluated.

Although phenotypical differences were observed among the *M. pachydermatis* strains, the results were not correlated with the origin of the strains nor the phylogenetic patterns. Nonetheless, multilocus phylogenetic analyses of *M. pachydermatis* strains, performed by the first time in this study, suggest that a speciation process where these yeasts would be adapting to different thriving conditions might be occurring.

Computational methods to explore viral species from wastewater metagenomics data.

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Many viral infectious diseases are transmitted by consumption or contact with water or food contaminated with the discharge of untreated or even treated wastewater. Treated wastewater is increasingly recognized as a resource of water nutrients and is reused in industry, for landscape irrigation, aquifer recharge and, especially in Mediterranean Europe, also for irrigation of fresh produce for human consumption. Water quality, is evaluated according to FIB levels (Fecal Indicator Bacteria), but these indicators do not always correlate with enteric viruses. Wastewater contains many potential well known pathogenic bacteria and viruses; but also other potential emerging bacterial and viral pathogens largely unknown. The use of molecular techniques can give us a clue when searching for a concrete pathogen but might therefore miss some others.

The introduction of new techniques such as metagenomics in the analysis of water can give us important information of all the viruses present in a sample at the same time. Our main objective is to apply metagenomics analyses for the study of viral richness, as well as to expand the list of known viruses present in urban sewage from Barcelona. For that, we have generated a customized protocol to analyze viral wastewater data from NGS (MiSeq Illumina); the parameters for the distinct bioinformatics tools used have been tuned to improve the detection of known and novel candidates. Main steps of this protocol include standard cleaning, filtering, alignment, and taxonomy classification. The metagenomics study using Illumina platform facilitated the description of more than 25 different viral families; among those, 9 are related to human viral pathogens. Members of the Caliciviridae family such as Human Sapoviruses and Noroviruses, several Human Astroviruses including some recombinant strains and recently described parvoviruses related to gastroenteritis have been found using NGS. Finally, the presence of the recently described virus *Salivirus/klassevirus*, a new genus belonging to *Picornaviridae* family, in urban sewage, has been confirmed by conventional RT-PCR.

Polyphosphate is Involved in Cell Cycle Progression and Genomic Stability in *Saccharomyces cerevisiae*

Samuel Bru, Joan Marc Martínez, Sara Hernández-Ortega, Eva Quandt, Javier Jiménez and Clotet J

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Polyphosphate (polyP) is a linear chain of up to hundreds of inorganic phosphate residues that is necessary for many physiological functions in all living organisms. In some bacteria, polyP supplies material to molecules such as DNA, thus playing an important role in biosynthetic processes in prokaryotes. In the present study, we set out to gain further insight into the role of polyP in eukaryotic cells. We observed that polyP amounts are cyclically regulated in *Saccharomyces cerevisiae*, and those mutants that cannot synthesise (*vtc4Δ*) or hydrolyse polyP (*ppn1Δ*, *ppx1Δ*) present impaired cell cycle progression. Further analysis revealed that polyP mutants show delayed nucleotide production and increased genomic instability. Based on these findings, we concluded that polyP not only maintains intracellular phosphate concentrations in response to fluctuations in extracellular phosphate levels, but also muffles internal cyclic phosphate fluctuations, such as those produced by the sudden demand of phosphate to synthesize deoxynucleotides just before and during DNA duplication. We propose that the presence of polyP in eukaryotic cells is required for the timely and accurate duplication of DNA.

Water treatments for Hepatitis E Virus: UV radiation and Flocculation-Chlorination sachets

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Hepatitis E Virus (HEV) is a major cause of waterborne outbreaks in areas with poor sanitation. As the fecal-oral route is the predominant mode of transmission for HEV, and there are currently no efficient curative therapies HEV, safe water supplies are the keystone for preventing HEV outbreaks, and data on the efficacy of disinfection treatments are urgently needed. We evaluate the ability of UV radiation and flocculation-chlorination sachets (FCSs) to reduce HEV in water matrices. The HEV-p6-kernow strain was replicated in the HepG2/C3A cell line to obtain viral suspensions, and we quantified genome number using qRT-PCR and infectivity using an immunofluorescence assay (IFA). UV experiments were performed with 253.7nm low-pressure lamps radiating viral suspensions in phosphate buffer by doses ranging from 0 to 1400 J/m². Flocculation-Chlorination sachets efficiency were tested with two commercial preparations (PUR Purifier of Water™ and WaterMaker™) along with two different water matrices (low and high turbid artificial waters).

UV irradiation tests using low-pressure radiation showed inactivation kinetics for HEV of 99.99% with a UV fluence of 232 J/m² (IC 95%, 195,02 – 269,18). Furthermore, evaluation of the kinetics of HEV inactivation by UV provide us valuable information for estimating infectivity based on qRT-PCR data. The FCSs preparations significantly reduced viral concentrations in both water matrices, performing better in low turbid waters with LRV mean values of 1.28 and 3.37 for HEV and MS2 respectively. Inactivation data obtained for HEV after UV radiation indicate that this emerging pathogen should be inactivated up to 99.99% following a fluence of 231.94 J/m² (195.02 – 269.18), which is below the radiation level already proposed in international guidelines (400 J/m²). FCS solutions for point-of-use disinfection achieved significant reductions in virus concentration, although the reductions were less than the 4.5 LRV recommended by WHO regulations.

A method for monitoring bacterial gene expression as determined by oxygen concentration

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Oxygen concentration is one of the main factors controlling bacterial physiology: it determines whether an environment could be acceptable for colonization by any specific bacterial species, and when bacteria can grow under different oxygenation conditions, they have to change most of their metabolism and gene regulation to adapt to the different situation.

Clinically relevant species, like the perilous opportunistic pathogen *Pseudomonas aeruginosa*, show their full virulence when growing as a biofilm, complex structures with clusters of cells encapsulated in an extracellular polymeric matrix, where they see increased their resistance to antibiotic therapies and physical stress, and that are essential for the development of chronic infections. In biofilms, oxygen cannot diffuse freely throughout the structure, generating an oxygen concentration gradient and leading to the presence of microaerophilic and anaerobic environments.

In this and many other examples, it has been studied how bacteria change their gene expression profiles aerobically and anaerobically. However, in real world conditions, transition from aerobiosis to anaerobiosis is never done in a single step, having always a spatial and/or temporal gradient of oxygen concentration; unfortunately, bacterial performance and gene regulation during intermediate stages of this gradient, in microaerophilic environments or during dynamical oxygen concentration changes, has not been studied properly.

In our lab we have developed a method to monitor bacterial gene expression as determined by oxygen concentration, during a progressive change in oxygenation conditions, that can be implemented in any microbiology lab and that only relies on common laboratory equipment. The method is based on a chemostat-like bioreactor coupled to an oxygen tension continuous detection system that also allows for discontinuous sampling. Initially, this system is now being applied to the determination of the changes in ribonucleotide reductase gene expression profile determined by oxygen concentration in a *Pseudomonas aeruginosa* liquid culture.

This work was supported in part through grants to ET from the Ministerio de Economía y Competitividad (BFU2011-24066 and BIO2015-63557-R), Generalitat de Catalunya (2014 SGR01260) and Catalan and Spanish Cystic Fibrosis foundations. LP is thankful to the Generalitat de Catalunya for its financial support through the FI programme (2015-FI-B-00817).

Virulence factors on *Mucor circinelloides* strains: a comparative study

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The dimorphic filamentous fungus *Mucor circinelloides* is able to infect immunocompromised patients causing fatal infections. Our experimental studies on a murine model showed that strain CBS277.49 cause 100% mortality after systemic infection by 5×10^6 CFU/mouse while no mortality was found after infection with strain NRRL3631, being both wildtype strains. Further phenotypical characterization of both strains revealed diversity related to spore size heterogeneity, spores viability, germination kinetics, chitin/chitosan rates and toxic compounds sensitivity. This seems to reflect an important variation among strains from the same species that may result from diverse genetic mechanisms. In order to determine the genetic basis of the fungal virulence of *M. circinelloides* we have approached genome-level analyses by Next Generation Sequencing method (NGS). Expecting that genome comparison between both strains could reveal specific genes candidates for virulence factors, we have re-sequenced and assembled the genome of NRRL3631 (Illumina) and performed a pairwise comparison with the CBS277.49 genome (JGI database) using Artemis Tool Comparison and read mapping for variant call analyses using Burrows-Wheeler Aligner. We have identified around 300 protein-coding genes not present in the avirulent strain and another 200 genes highly polymorphic or partially absent. We have also identified 176.188 homozygous SNPs distributed across the 26 scaffolds and 16.086 small indels. Among the functional annotated genes, we highlight differences in genes-related to hydrolytic enzymes, membrane transport, regulatory, extracellular and cell wall biogenesis however, most of the differences were found in protein-coding genes with unknown function. Currently, we are focus on extracellular proteins which are being investigated in detail by knock-out mutants' in aim to determine their biological role in pathogenesis.

Predictive microbiology as an emerging approach for assessing microbial inactivation by high pressure processing

Sara Bover-Cid, Anna Jofré, Cristina Serra and Margarita Garriga

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High pressure (HP) processing of food is usually applied to enhance microbiological food safety and extend shelf-life. Product formulation with organic acids, as antimicrobial agents (AMA), may affect the lethality of HP. Mathematical modeling enables the quantitative characterization of microbial behavior and contributes to a better understanding of the effect of food preservation strategies. The aim of the present study was to model the effect of lactate and diacetate on the HP inactivation of *L. monocytogenes* in cooked ham.

Cooked ham batches were manufactured with: 1.4% potassium lactate, 2.8% potassium lactate, 0.1% sodium diacetate and 1.4% lactate plus 0.1% diacetate. Once sliced, products were inoculated with *L. monocytogenes* (three different strains, i.e. CTC1011, CTC1034 and ScottA, independently). Vacuum-packed samples were pressurized at 400 MPa for different holding times (0-10 min). *L. monocytogenes* survival was monitored by enumeration on chromogenic medium. Inactivation kinetics was evaluated by fitting primary models to the survival data obtained by each product formulation and strain. Secondary modeling was applied to quantify the effect of lactate concentration.

The HP-inactivation kinetics depended on both the product formulation as well as the *L. monocytogenes* strain. Overall, the presence of lactate caused a piezo-protection as the inactivation rate was lower in cooked ham formulated with lactate than in the control product. The higher the lactate concentration, the lower the inactivation rate. By contrast, diacetate slightly enhanced HP inactivation of *L. monocytogenes*. A notable difference was denoted in the primary kinetic behavior among the studied strains. A shoulder shape was observed for the food isolates (CTC1011 and CTC1034), while a tail was recorded for the clinical isolate (ScottA).

The design, validation and implementation of high pressure processing requires a tailor-made approach, taking into account the specific product formulation and also selecting the most appropriate strain/s for process validation.

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Phage therapy and resistance to bacteriophages

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As consequence of the increase of bacterial resistance to antimicrobial agents, phage therapy has emerged as a realistic alternative to antimicrobials. Bacteriophages have proved to be interesting therapeutics agents due to their special characteristics: target specificity, rapid bacterial killing and self-replication in presence of their bacterial host.

Nevertheless, bacteria and their bacteriophages are under the effect of a parasitic relationship in which both evolve to survive. So, bacteria can evolve to avoid the action of the phages becoming resistant to them by different mechanisms, jeopardizing the success of the phage therapy. However, in this regard, a lower fitness of the bacterial cells resistant to bacteriophages has been suggested (1, 2). Nevertheless, the resistance to bacteriophages is a conflictive point that must be assessed in the frame of the phage therapy.

In this work, this subject has been studied in a model of oral bacteriophage therapy (3). Hence, commercial broilers infected with *S. typhimurium* were treated with a bacteriophage cocktail composed by three virulent bacteriophages (4, 5, 6) whose attachment site is the *Salmonella* lipopolysaccharide (LPS). Even though the emergence of bacteriophage-resistant *Salmonella* during phage therapy could be expected, in this study, none of the isolates from treated broilers was resistant to bacteriophages. This result could be influenced by the concomitant effect of two aspects: i) the significant and high reduction of the *Salmonella* population due to the treatment with the bacteriophages (3), and ii) the lower fitness and persistence described for the resistant cells in the cecum of infected broilers (1).

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Application of ERIC-PCR genotyping method for screening the prevalence of three cryptic species uncover by *Aeromonas media* clinical and environmental strains

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Aeromonas media described in 1983 by Allen in river freshwater and have been found to be very abundant in reclaimed wastewater. In addition it acts as an opportunistic emerging pathogen causing a wide spectrum of diseases in human and animals. Previous studies showed that this species is highly heterogenous, because strains from different origins group in three clades (A, B and C) on the basis of their pattern of enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), the average nucleotide identity and the in silico DNA-DNA hybridization. Clade B included the type strain of *Aeromonas media* and could be differentiated from the others by the DL-lactate utilization and cefoxitin resistance. The clade A, embraced what we though was a new species, and in fact a Spanish group recently proposed the new species recovered from river water as *Aeromonas rivipollensis* that clustered perfectly with the strains included in the clade A. The clade C was proposed as "*Aeromonas paramedia*" waiting to obtain a larger number of strains for making the species description. The main objective of this study was to characterize by ERIC-PCR the prevalence of these three cryptic species using 224 isolates of *Aeromonas media* recovered from clinical (n=13) and environmental (n=211) samples. As indicated above the three clades could be recognized after ERIC-PCR genotyping by the presence of specific combinations of bands. *Aeromonas rivipollensis* showed the band of 650 and 750 bp while *Aeromonas media* showed bands at 650 and 500 bp and "*Aeromonas paramedia*" showed specific bands at 500 and 900 bp. The overall results independently of the origin of the strains showed that *Aeromonas rivipollensis* was the most frequent species (64.73%) followed by *Aeromonas media* (31.69%) and "*Aeromonas paramedia*" (3.58%) that was the only one not detected among the clinical strains. This study provide 8 additional of "*Aeromonas paramedia*" and these results show that this species seems to more prevalent in environmental than in clinical samples, the latter being the origin of the only two existing strains of this potential new species. Further studies using a higher number of clinical strains may help to clarify their true prevalence in the clinical setting.

A continuous microalgal photobioreactor for emerging contaminants removal in urban wastewater: set up and community study

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Emerging contaminants in wastewaters are nowadays rising concern due to their unknown potential to affect natural ecosystems. The presence of these contaminants in local wastewaters is a fact, and conventional wastewater treatment plants (WWTP) are not suited for their proper removal. To address this issue, a continuous microalgal photobioreactor (PBR) was set up to evaluate the capacity of phototrophic microorganisms, in association with heterotrophic bacteria, for emerging contaminants removal. The PBR was fed with urban wastewater, during the first period the hydraulic retention time (HRT) was 8 days, while during the second period it was increased to 12 days. The main parameters monitored were temperature, total suspended solids, pH, dissolved oxygen, total carbon, ammonia nitrogen, total phosphorus and chemical oxygen demand. Most of the parameters were measured from both the wastewater and the PBR.

Microalgal growth was in accordance with temperature, solar irradiation and chemo-physical conditions monitored. Moreover, the nutrient removal rates obtained from the PBR activity met the requirements established by the current European commission directive 98/15/15EC. Pharmaceuticals removal was also assessed during the two operating periods and promising removal rates (from 30 to 90%) were observed for most of the detected compounds. Finally, microbial communities (Eukarya and cyanobacteria) were studied by denaturing gradient gel electrophoresis (DGGE), a PCR-dependant molecular fingerprinting technique. Prominent bands from the DGGE were re-amplified and sequenced in order to know their taxonomical affiliation.

Enhanced recovery of *Arcobacter* spp. using NaCl in culture media and re-assessment of the traits of *Arcobacter marinus* and *Arcobacter halophilus* isolated from marine water and shellfish

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The genus *Arcobacter* is a relatively poorly known group of bacteria, and the number of new species and sequences from non-culturable strains has increased considerably in recent years. This study investigates whether using media that contain NaCl, might help to improve the recovery of *Arcobacter* spp. from marine environments. To this aim, 62 water and shellfish samples were analyzed in parallel, with both the previously used culture method (enrichment in Arcobacter-CAT broth followed by culture on Blood Agar) and with a new one consisting of supplementing the Arcobacter-CAT enrichment broth with 2.5% NaCl (w/v) followed by culture on Marine Agar. The new approach yielded ca. 40% more positive samples than the previously used method and provided a higher diversity of known (11 vs. 7) and unknown (7 vs. 2) *Arcobacter* species. Among the 11 known species recovered using the media with NaCl, *Arcobacter marinus* and *Arcobacter halophilus* were isolated only by this method. These two species have never before been isolated after their description that in both cases included only a single strain. Considering this, the phenotypic characteristics of these species are re-evaluated using the new strains. Strains of *A. halophilus* gave the same phenotypic profile as the type strain. However, some strains of *A. marinus* differed from the type strain in that they did not hydrolyse indoxyl-acetate. Therefore, *A. marinus* becomes the first *Arcobacter* species to show a varying ability to hydrolyse indoxyl-acetate. This study shows to which extent a simple variation to the culture media can have a big influence on the positive samples and on the community of species recovered.

Of how stress affects bacterial collective behavior

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The motility over a surface is an essential property shown by the majority of bacterial pathogens that has been associated with virulence and elevated resistance to multiple antibiotics.

Recently our group has demonstrated that bacterial cells adapt their surface motility in response to the presence of DNA-damaging agents by sensing these compounds via SOS system activation. The SOS-response is an inducible genetic pathway governing DNA damage repair. It is controlled by two key proteins, the LexA repressor and the RecA inducer. When during surface colonization DNA injuries are generated, RecA activates the SOS machinery by inducing LexA auto-cleavage and so DNA-damage repair systems. Further, SOS response activation also generates an increase of RecA concentration, since *recA* is one of the first genes to be induced in the hierarchy of this system. During the time that SOS machinery remains active, the high amount of intracellular RecA disturbs its equilibrium with CheW protein, an essential anchorage protein of the chemotaxis pathway. CheW has been thoroughly described to play a key role in surface motility behavior causing the cessation of swarming through its interaction with RecA. When SOS response is activated, RecA protein hijacks CheW, decreasing its availability to anchor CheA histidine kinase to the chemoreceptors that are located in the cell poles, thus avoiding the self-assembly of the chemotaxis sensory array. Due to RecA-CheW pair formation, the distribution of CheW among the cells changes from the cell poles to several organized *foci* along the cell in helically pattern similar to that of other proteins associated to cytoskeleton and thus altering the swarming ability. If there is not enough CheW protein to generate the sensing basal unit, signaling array cannot be assembled, and so, the cell loses its ability to swarm. This control mechanism is reversible, the repair of the DNA damage leads the repression of the SOS system, restoring the intracellular balance between RecA and CheW, recovering polar signaling array assembly and therefore also swarming ability.

Concluding, our model proposes a general response mechanism for bacterial cells that make use of this specific mechanism driven by a single signal to respond to a broad range of DNA-damaging agents during niche colonization and adaptation.

Next generation sequencing as a tool to diagnose new potential viral causes in Non-A-E hepatitis patients

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The etiological agent of clinical hepatitis, identified by its distinguishing yellow jaundice, was found to be infectious in the early 1900s. Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. However, to surprise of many, a substantial number of patients with hepatitis remain of unknown etiology. A variety of new molecular cloning techniques such as sequence-independent, single-primer amplification and representational difference analysis led also to the discovery of hepatitis E (HEV) and the GB virus C and to the TT virus. We analyse serum of patients with clinical signs of hepatitis (High ALT, AST, TB and DB) using metagenomics for the study of viruses. Serum samples were studied in five pools: two of men and one of women with hepatitis without an identified etiological agent, one pool of patients with history of autoimmune/immunosuppressed disease, and in the last pool patients with positive diagnose of HEV. The nature of the genome of the viruses found were ssDNA and ssRNA(+). In all the pools were found virus ssDNA, mainly Torque Teno viruses, Torque teno mini Viruses and Torque Teno midi viruses, all belongs to the family Anelloviridae. In one pool of men without cause and in HEV pool we found GB virus C, we identify one patient with this virus by nested PCR and sequencing analysis of the amplicon, the patient had history of chronic hepatitis with periodic recurrences. In the HEV pool, HEV was studied and 24 contigs of HEV virus with sizes between 239 and 1571 bp were analysed. NGS will be used as a preliminary step to identify potential causal agents that will be confirmed by epidemiologic studies.



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PÒSTERS

Pòster 1

In vivo* efficacy of antifungal combination therapies against *Aspergillus fumigatus

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Aspergillus fumigatus is the most common mold causing invasive fungal infection in immunocompromised patients, especially in those with hematological malignancies. Voriconazole (VRC) is the first-line therapy for the treatment of aspergillosis, but, unfortunately, the therapeutic failure is high. For this reason, an exploration of alternative strategies against this difficult-to-treat infection is crucial. Combinations of antifungal agents are not common therapies but might be good alternatives for infections by resistant organisms or when the standard treatments fail. The goal of this study was to evaluate the efficacy of the combinations of posaconazole (PSC) with amphotericin B (AMB) or anidulafungin (AFG) against three isolates of *A. fumigatus* in a murine model of disseminated aspergillosis, comparing the results with those of the corresponding monotherapies and VRC. Groups of 16 immunosuppressed male OF-1 mice were challenged with 10^4 colony forming units (CFU) of each *A. fumigatus* strain via the lateral vein of the tail. The treatments started one day post-infection and lasted 7 days, and consisted on: 0.8 mg/kg of AMB i.v. once daily (QD), PSC at 20 mg/kg orally by gavage (p.o.) twice a day (BID), AFG at 10 mg/kg i.p. QD, VRC at 25 mg/kg p.o. QD or the combinations of PSC plus AMB or PSC plus AFG at the commented doses. After the last dose, animals were euthanized and kidneys were removed and homogenized for CFU/g of tissue determination. Both combined therapies increased survival of mice in comparison with control groups, and in some cases improved the efficacy of monotherapies with PSC, VRC and AMB. In terms of fungal burden, all treatments reduced fungal burden with respect to the control and additionally, both combinations sterilized kidneys of practically all the infected animals, suggesting that this combination deserves to be explored as alternative for the treatment of aspergillosis refractory to the first-line therapy.

Pòster 2

In vitro* data are predictive of the *in vivo* efficacy of voriconazole in murine infection by *Candida glabrata

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Invasive candidiasis (IC) is an important cause of morbidity and mortality despite current antifungal treatment, being *C. albicans* the most common species involved in human infection. However, non-*albicans* species such as *Candida glabrata* have increased its incidence being currently the second most frequent bloodstream fungi causing 10-20 % of IC in immunocompromised patients. In addition to the increased penetrance of *C. glabrata* into the immunocompromised population, it is remarkable the emergence of multidrug resistance isolates making *C. glabrata* an interesting object of study. Echinocandins are the recommended therapy in neutropenic patients against IC but based on different clinical data, voriconazole (VRC) seems an option as initial treatment of IC in febrile neutropenic patients however, there are no experimental data on the efficacy of this drug against IC by *C. glabrata*. Our aim was to evaluate if the proposed epidemiological cut-off value (ECV) i.e., 0.25µg/mL, is predictive of efficacy in a murine model of disseminated infection using 27 clinical isolates with different minimum inhibitory concentrations (MICs). *In vitro* activity of strains was determined by triplicate according to the document M27-A3 by CLSI. For *in vivo* study we used groups of 16 mice, immunosuppressed 2 days prior to infection by 200 mg/kg of cyclophosphamide intraperitoneally. On the day of infection, animals were challenged with 2x10⁸ CFU/animal intravenously and 24 h after infection animals received VRC at 40 mg/kg once daily during 7 days. The efficacy of the treatment was evaluated by tissue burden studies in kidneys. VRC reduced the fungal tissue burden in mice infected with strains showing MICs below the ECV (9 strains). Variable efficacy was observed when the MIC equalled the ECV (3 strains), and inefficiency when the MIC exceeded the ECV (15 strains). These results support the proposed ECV as starting point for predicting successful outcome in VRC-based therapies against IC by *C. glabrata*.

Pòster 3

MALDI-TOF and *rpoB* sequencing reveals previously undetected case of *Arcobacter butzleri* from human diarrhoea

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The genus *Arcobacter* includes aerotolerant gram-negative bacteria and was the fourth most common pathogenic bacteria genus isolated from stool specimens of patients with acute enteritis in Belgium when using a specific culture media. However, this is not the case in many other studies where *Arcobacter* is identified by chance from media used for *Campylobacter* or other enterobacteria. The objective of the present study was to characterize the sequence types and virulence genes present in *Arcobacter butzleri* strains associated to cases of human diarrhea. Eight *A. butzleri* strains, recovered from CIN agar (BD) and Campylosel agar (bioMérieux) and identified by MALDI-TOF and *rpoB* gene sequencing were investigated. Sequence types were determined after sequencing the *aspA*, *atpA*, *glnA*, *gltA*, *glyA*, *pgm* and *tkt* genes by comparison with the MLST database. The presence by PCR of 5 virulence genes (*ciaB*, *cadF*, *cj1349*, *hecA* and *irgA*) was determined using primers described previously. The 8 *A. butzleri* strains were all confirmed as this species with the sequences of housekeeping genes. 7 strains showed to belong to new sequence types (ST) on the basis of the new alleles found for the genes *glyA* and *tkt* despite alleles found for *atpA* and *glnA* were already present at the database. The remaining ST already existed in the database (ST2) and corresponded to two human clinical isolates from Belgium and France. The new allele found in *glyA* gene (allele 517) was present in three of our strains. The 8 strains cluster with strains of the database isolated from human stool and meat or animal origin. All the strains possessed *ciaB* and *cj1349* genes, while *hecA* and *irgA* were never detected. Isolation of new sequence types of *Arcobacter butzleri* from CIN agar seems to be common and MALDI-TOF enabled their fast and reliable identification.

Pòster 4

Microbiological and enzymatic characterization of pregnant mare's milk. Evaluation of its probiotic, antimicrobial and preservative properties

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ABSTRACT

Mare's milk is a traditional food in Central Asia, Mongolia and the old Soviet Union countries, where it has been an important source of food for their populations. Due to its high quality, there has been a rise in the consumption of mare milk in Europe. Its content in carbohydrates, proteins and salts, as well as its casein's profile, is more similar to human milk than cow milk. The objectives of this research are (1) the mare's milk microbiological and enzymatic characterization, (2) the evaluation of potential probiotic and antimicrobial capacity of lactic acid bacteria isolated, and (3) the assessment of this milk as a protector for freeze drying processes and long-term preservation of lactic strains.

The results confirmed that the mare's milk has a broad microbiome that is modified, quantitatively and qualitatively, throughout gestation. It has also been seen as containing a variety of constitutive enzymes which have a quantitative variability during pregnancy. Of the 11 strains of lactic acid bacteria isolated from mare's milk, six showed a potential use as probiotics because of their safety and their resistance to in vitro gastrointestinal conditions. The lactic acid bacteria isolated from equine milk and faeces have an antimicrobial activity against a variety of undesirable microorganisms as *Kocuria rhizophila*, *Pantoea* sp., *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus* spp., *Listeria monocytogenes*, *Enterococcus faecalis* and *Proteus vulgaris*. Its antimicrobial action is mainly due to the formation of organic acids, but also the production of hydrogen peroxide and thermolabile compounds.

The mare's milk powder is presented as a good alternative to the use of cow's milk for the freeze drying of *Lactobacillus* spp. and *Lactococcus lactis* equine strains. Due to its composition, very similar to human milk, it can be a good excipient for dietary supplements and functional foods.

Pòster 5

Microbiological quality of olives and antimicrobial action

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The aim of this work was to proceed the microbiological characterization of olives and to verify its antimicrobial action and of compounds extracted from the pulp (maslinic acid, oleanolic acid, oleuropein, tyrosol and hydroxytyrosol). Olives evaluated were *Sevillana*, *Arbequina*, *Marfil* and *Empeltre*. For the microbiological quality counts were performed of lactic acid bacteria (LAB), *Staphylococcus aureus*, coliforms and other enteric pathogens, moulds and yeasts, *Clostridium perfringens*, *Salmonella* spp., *Listeria monocytogenes*, and a several pathogens microorganisms. The antimicrobial activity was observed in 96 wells plates and microorganisms (*Lactobacillus brevis*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Aspergillus niger*, *Penicillium rugulosum*, *Candida albicans* and *Saccharomyces cerevisiae*) were treated with olive suspensions and its compounds. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) were evaluated. The moulds and yeasts were analyzed with disc diffusion in agar method. *Sevillanas* olives had growth of 6.6×10^6 UFC/g of LAB and 8.7×10^2 UFC/g of *S.aureus*. Arbequinas and Marfil olives showed growth of coliforms and other enteric pathogens (8.9×10^7 and 4.0×10^7 UFC/g, respectively) and moulds and yeasts growth in all olives. It was not detected *C. perfringens*, *Salmonella* spp. or *L. monocytogenes* in any olives analysed. Tyrosol and hydroxytyrosol doesn't inhibit the growth of microorganisms studied. Oleuropein inhibit the growth of *S. enteritidis*, *S. thiphymurium* and *Pseudomonas*. Maslinic acid and Oleanolic acid showed growth inhibition of all microorganisms analysed, but *L.brevis*, *L.plantarum* and *B.subtilis* showed MBC. The results showed that is necessary improve the quality and safety of olives using good practices in agriculture, hygiene and manufacturing. No olive extract was able to completely reduce the growth of microorganisms. *Sevillanas* olives stimulated the growth of LAB. This may be due to the type of treatment used or the olives would be acting as prebiotic, stimulating the growth of *Lactobacillus* spp.

Keywords: olives; microbiological quality; antimicrobial action; maslinic acid; oleanolic acid; hydroxytyrosol

Pòster 6

Determinació de compostos volàtils orgànics de cultius fúngics mitjançant SPME-GC-MS

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Els compostos orgànics volàtils (VOCs) són molècules de baix pes molecular elaborades per diferents gèneres de fongs i que poden actuar com a indicadors de la seva activitat. En aquest estudi s'ha realitzat la identificació dels VOCs produïts per cultius de *Verticillium dahliae*, *Trichoderma viride* i *Penicillium rugulosum* mitjançant tècniques basades en l'adsorció dels VOC mitjançant microextracció en fase sòlida (SPME), posterior separació amb cromatògraf de gasos (GC) i identificació mitjançant espectrometria de masses (MS).

Per a l'extracció dels VOC s'ha utilitzat una fibra SPME DVB/CAR/PDMS capaç d'adsorbir compostos aromàtics, volàtils i semi-volàtils amb un pes molecular de 40-275 DA del *headspace* del cultiu fúngic. Per la separació dels VOC s'ha treballat amb una columna capil·lar de 30 m de llargària, 0.25 mm de diàmetre i 0.25 µm de diàmetre de fibra. El gas transportador utilitzat ha estat Heli a un flux constant de 38 cm/segon. Per la detecció dels VOCs s'ha utilitzat un espectròmetre de masses amb quadrupol i han estat identificats mitjançant les llibreries de referències Wiley i NIST.

Amb aquest assaig s'han identificat més de 100 VOCs per cada cultiu, entre els que destaquen: (1) VOCs del Complex C8 comuns entre diferents gèneres de fongs i que indiquen que els cultius estan en fase de creixement, com per exemple 1-octen-3-ol i 3-octadien; (2) altres components comuns no pertanyents al Complex C8, com el 2-butanol, 1-propanol i el phenol; i (3) VOCs propis de cada gènere, alguns d'ells interessants per diferents tipus d'indústria, com l'azulene (producció de pigments), la thiazolamina (producció d'antifúngics), la juniper camphor (elaboració de perfums) i el menthol (indústria alimentària i farmacèutica).

Pòster 7

Microbiología del caracol *Helix aspersa* Müller, y aplicaciones biotecnológicas para su mejoramiento sanitario con impacto en su comercialización

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RESUMEN

Línea de investigación dedicada principalmente al mejoramiento sanitario de granjas de caracoles, mediante la aplicación de un probiótico como alternativa biotecnológica.

La investigación se ha dirigido en dos líneas principales: i) optimizar a escala de laboratorio el rendimiento productivo de creación de biomasa probiótica; ii) estudios in vivo de la aplicación del probiótico en granjas de caracoles ubicadas en la comunidad autónoma de Catalunya

Como logros principales hasta la fecha, está el mejorar significativamente el crecimiento de la cepa probiótica lo que permite obtener biomasas probióticas con alta concentración del microorganismo ($12 \log_{10}$ ufc/mL), así como de disponer de otro crioprotector de base no láctea como *carrier* del proceso de liofilización. Estos resultados han permitido trabajar en la optimización de las vías de inoculación sobre los animales, para lograr asegurar el efecto probiótico sobre los caracoles.

A nivel de estudios con las granjas de caracoles, se dispone de contundente información de la microbiota presente en caracol *Helix aspersa* Müller, así como un panorama de los problemas de contaminación presentes en este rubro productivo. Esta información ha sido importante para tomar medidas y estrategias para el control de contaminación, con el objetivo de obtener animales sanos, y cerciorar un que el alimento sea seguro, ya que finalmente este va destinado al consumo humano.

Pòster 8

Deciphering the involvement of ribonucleotide reductase in adherent-invasive *Escherichia coli* LF82 infection

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A critical step in the life cycle of all organisms is the duplication of the genetic material during cell division. Ribonucleotide reductases (RNRs) are essential enzymes for this step because they control the *de novo* production of the deoxyribonucleotides required for DNA synthesis and repair. *Enterobacteriaceae* have three functional classes of RNRs (Ia, Ib and III), which are transcribed from separate operons and encoded, respectively by the genes *nrdAB*, *nrdHIEF* and *nrdDG*. Here, we investigated the role of RNRs in the virulence of adherent-invasive *E. coli* (AIEC) isolated from Crohn's disease (CD) patients. Interestingly, the LF82 strain of AIEC harbors four different RNRs (two class Ia, one class Ib and one class III). Although the *E. coli* RNR enzymes have been extensively characterized both biochemically and enzymatically, little is known about their roles during bacterial infection. We found that RNR expression was modified in AIEC LF82 bacteria during cell infection, suggesting that RNRs play a crucial role in AIEC virulence. Knockout of the *nrdR* gene, which encodes a transcriptional regulator of RNRs, decreased AIEC LF82's ability to adhere to intestinal epithelial T-84 cells, to interact with the ileal mucosa using ileal loop modeling and to colonize the gut mucosa of transgenic mice that express human CEACAM6. Microarray experiments demonstrated that NrdR plays a crucial role in AIEC virulence by interfering with bacterial motility and chemotaxis. Thus, the development of drugs targeting RNR classes could be a promising new strategy to control gut colonization by AIEC bacteria in CD patients.

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Pòster 9

***Pseudomonas aeruginosa* exhibits deficient biofilm formation in the absence of class II and III ribonucleotide reductases due to hindered anaerobic growth**

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Most chronic infections in humans are caused by communities of microorganisms living in organized structures, known as biofilms. Biofilm-related infections, such as pneumonia (in patients suffering for cystic fibrosis –CF- or chronic obstructive pulmonary disease –COPD-) and catheter-associated infections, affect millions of people in the developed world. Nowadays, the common antibiotics cannot use to treat the biofilm. Since, cell clusters in biofilms are characterized by presenting, in its extracellular polymeric matrix, gradients of oxygen, nutrients and metabolic waste products. The so-formed chemical heterogeneity (e.g., the presence of anoxic areas) leads to the appearance of different metabolic activities that give antibiotic resistance.

In this work we have studied *Pseudomonas aeruginosa*, as a model bacterium for biofilm research. It causes biofilm-related chronic infections and presents high metabolic versatility, together with an extreme antibiotic resistance. Our work is focused in an essential enzyme for *P. aeruginosa* growth, the Ribonucleotide Reductase enzyme (RNR). These enzymes catalyse the reduction of ribonucleotides (NTPs) to their corresponding deoxyribonucleotides (dNTPs), thereby providing the building blocks for DNA synthesis. There are three different RNR classes (class I, class II and class III), which are, oxygen-dependent, oxygen-independent and oxygen-sensitive respectively (1). Class II and III RNR are essential for anaerobic growth in *P. aeruginosa*, and we probe in this work the importance during biofilm formation, and therefore can be considered an ideal targets for new therapies against *P. aeruginosa* chronic infections.

1). Torrents, E. (2014). Ribonucleotide reductases: essential enzymes for bacterial life. *Frontiers in Cellular and infection microbiology*. 4:52. doi: 10.3389/fcimb.2014.00052.

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Pòster 10

Improving wound healing through infection treatment

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Wounds, which are caused by physical breaks in the epithelium, represent a very common and serious health problem worldwide, especially those that turn into chronic wounds. The exposure of the internal tissue to the effect of external environment promotes pathogenic bacteria to colonize the infection site, delaying the wound healing processes of the host tissue and leading, in some cases, to chronic wounds.

Bacteria infecting wounds grow forming complex polymicrobial communities in a biofilm-like lifestyle. Such complex structure evades easily the host immune response and is highly resistant to the antibiotics action.

So as to allow wound-healing process in wounds, it is important to develop healing therapies to allow tissue regeneration but also the eradication of the infection, which is the factor that induces the chronicity in wounds.

Here, we are focusing on new wound healing strategies in the perspective of wound infections, specifically fighting bacterial biofilm infection in wounds using an *in vitro* wound biofilm model that mimics natural conditions present in wounds.

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Pòster 11

Antimicrobial effect of atmospheric DBD plasma onto packaged solid samples with different atmospheres

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The use of non-thermal technologies in microbial decontamination is a key area of interest for industry since they produce no temperature damages to products or materials. Among non-thermal technologies, atmospheric Dielectric Barrier Discharge (DBD) plasma has several advantages. It can work under atmospheric conditions and have extremely flexible geometry and configurations. In the present contribution, the potential of atmospheric DBD plasma as an antimicrobial technology for in packaged solid samples decontamination was evaluated. In addition, the effect of the type and volume of gas contained inside the package during the plasma treatment was also studied. Membrane filters were employed as model solid samples and were inoculated with microbial cultures of *Escherichia coli* and *Aspergillus brasiliensis* at concentrations of 10^2 to 10^8 CFU/cm². Samples were packaged under two different gases, air or argon (a non-reactive inert gas) in two different size packaging, 150 and 300 cm³. Treatment time was established in previous test and was fixed at 60s. All experiments were performed in triplicated. Results obtained show that DBD plasma applied onto packaging samples have high effectivity in microbial decontamination. It is possible to reduce 10^7 CFU/cm² of *E.coli* in both packaging gasses with independence of the packaging size and 10^4 CFU/cm² of *A.brasiliensis* in samples packaged with air. These results conclude that DBD plasma is highly effective in microbial decontamination of air packaged samples and could potentially be used as a new antimicrobial technology with innumerable industrial applications.

Pòster 12

Microorganismes I Patrimoni cultural

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Línia d'investigació que té per objectiu l'estudi dels microorganismes que actuen sobre el Patrimoni cultural exercint dos tipus d'accions fonamentals: 1.- Alteració i/o destrucció; 2.- Bioremediació i/o consolidació.

1.- Alteració i / o destrucció. Es procedeix a l'aïllament i avaluació dels microorganismes presents en documents, escultures, pintures, gravats, etc. per posteriorment avaluar els possibles sistemes de neteja i tractament de les obres així com el seu seguiment al llarg del temps.

2.- Bioremediació i/o consolidació. S'investiga l'aplicació controlada de microorganismes sobre escultures, fòssils, edificis, etc., amb la finalitat de que afavoreixin l'eliminació dels microorganismes no desitjables i en determinades materials o afavoreixi la seva consolidació.

Paral·lelament s'avalua el possible paper dels microorganismes detectats en la manifestació i prevalença de processos patològics de diversa índole entre les persones en contacte amb obres alterades per microorganismes i la contaminació ambiental present.

Pòster 13

Microbiota de leche de yegua: efectos beneficiosos para la salud humana y animal

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Línea de investigación centrada en el estudio general de la microbiota procedente de la leche de yegua, así como los posibles efectos beneficiosos que éstas puedan aportar tanto a la salud humana como a la salud animal.

La investigación está estructurada en 3 objetivos:

- Control de calidad microbiológica de la leche de yegua.
- Estudio de la variación de la población microbiológica de leche de yegua.
- Aislamiento e identificación de microorganismos, como bacterias del ácido láctico, procedentes de la leche de yegua con posible capacidad probiótica.

Como estudio principal se llevó a cabo la administración de una bacteria ácido láctica de origen animal en gatos con gingivoestomatitis crónica felina (GECF). Además estos gatos padecían de alguna patología inmunosupresora, como el virus de la leucemia felina (FeLV) o el virus de la panleucopenia felina (VPF) consideradas como enfermedades que incrementan la probabilidad de padecer de GECF.

Como resultado se observó que la administración del probiótico en los gatos inmunodeprimidos afectados por GECF supuso una mejoría en cuanto al aumento del tiempo de aparición de recidivas. Durante la administración del probiótico se observaron signos de recidivas transcurridos 2 meses a diferencia de recaída a las 2 semanas o el mes mediante tratamiento farmacológico. Con este resultado además se pudo llevar a cabo la reducción de la frecuencia de tratamiento con antiinflamatorios y antibióticos para la GECF.

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