



SIMGBM
Società Italiana di
Microbiologia Generale
e Biotecnologie Microbiche

MICROBIOLOGY 2025

XXXV SIMGBM Congress

University Roma Tre
September 17-20



Programme and abstracts

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Programme

Wednesday, 17th September 2025

(Rectorate, Via Ostiense 133, Tower A, Main Hall)

14.00 Registration

15.40-16.00 Welcome and introductory information

16.00-17.00 | OPENING LECTURE (Supported by FEMS)

Chairs: **P. Landini** and **M. Ventura**

Jan Michiels (*VIB, Center for Microbiology, KU Leuven, Belgium*)

Antibiotic persistence: exploring mechanisms and evolutionary pathways

17.00-19.00 | PLENARY SESSION 1

Planetary microbiology: the role of microorganisms in a changing earth

Chairs: **E. Tamburini** and **L. Vezzulli**

17.00 **Marcela Hernández** (*University of East Anglia, UK*)

Microbiology of volcanic gases and the metabolism of one carbon compounds

17.35 **Samuel Chaffron** (*University of Nantes, France*)

Community network models to reveal marine plankton systems ecology and evolution

18.10 **Donato Giovannelli** (*University of Naples Federico II, Italy*)

Geosphere-biosphere coevolution: the role of trace metal availability in the evolution of biogeochemistry

18.35 **Paola Quatrini** (*University of Palermo, Italy*)

The role of the soil microbiota in mitigating desertification in Southern Europe

19.00 Welcome reception

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Thursday, 18th September 2025

(Dept of Law, Via Ostiense 159 - Main Hall and Hall 8)

09.00-11.00 | PLENARY SESSION 2**Multidisciplinary approaches to tackle antibiotic resistance**Chairs: **S. Buroni** and **E. Perrin**

- 09.00 **Mattia Zampieri** (*University of Basel, Switzerland*)
Unlocking new drug modes of action by multidimensional high-throughput metabolic profiling
- 09.35 **Francesco Imperi** (*University of Roma Tre, Italy*)
Genetics and evolution of polymyxin resistance in *Pseudomonas aeruginosa*
- 10.00 **Marco Fondi** (*University of Florence, Italy*)
Microbial interactions and antibiotic resistance
- 10.25 **Santiago Ramón-García** (*University of Zaragoza/ARAID Foundation, Spain*)
Novel *in vitro* tools to inform pre-clinical and clinical progression of tuberculosis combination therapy

11.00 Coffee Break

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11.30-13.30 | PLENARY SESSION 3**New frontiers in human and animal microbiome research**Chairs: **F. Turrone** and **C. Viti**

- 11.30 **David Berry** (*University of Vienna, Austria*)
Development of the gut microbiota-immune-brain axis in extremely premature infants
- 12.10 **Carlotta de Filippo** (*CNR, Institute of Agricultural Biology and Biotechnology, Pisa, Italy*)
Gut microbiota drives colon cancer risk associated with diet: a comparative analysis of meat-based and pesco-vegetarian diets
- 12.30 **Gabriele Andrea Lugli** (*University of Parma, Italy*)
Bifidobacteria as a model microbial group of the human gut microbiome
- 12.50 **Matteo Daghio** (*University of Florence, Italy*)
Polyphenol-rich by-products to modulate the activity of rumen microbiota
- 13.10 **Emilia Ghelardi** (*University of Pisa, Italy*)
In vitro models as cutting-edge tools for studying the gut microbiota: from static bioreactors to pocket dynamic platforms

13.30 Lunch and Posters viewing (odd numbers)**15.00-15.50 | PLENARY LECTURE 1** (Supported by FEMS)Chairs: **B. Colonna** and **P. Visca****Eric Cascales** (*Institut de Microbiologie de la Méditerranée, Aix Marseille Univ, Centre National de la Recherche Scientifique, Marseille, France*)

Architecture, assembly and mechanism of action of an antibacterial speargun: the type VI secretion system

15.50-17.10 | PLENARY SESSION 4**“Survive, escape, and kill”: mechanisms of host-pathogen interactions**Chairs: **G. Prosseda** and **L. Leoni**15.50 **Giovanni Delogu** (*Università Cattolica del Sacro Cuore, Rome, Italy*)
Host directed therapies for mycobacterial infections16.10 **Marco R. Oggioni** (*University of Bologna, Italy*)
Not macrophages but sinusoids capture bacteria in the human spleen16.50 **Vittorio Venturi** (*ICGEB, Trieste, Italy*)
LuxR solos represent a major family of cell-cell signaling regulators in the plant microbiome17.10 **Arianna Tavanti** (*University of Pisa, Italy*)
Fatal Attraction: role of adhesins in the virulence of the opportunistic pathogen *Candida parapsilosis*

THURSDAY

17.30 Coffee Break**18.00-19.00 | PLENARY LECTURE 2**Chairs: **E. Ricca** and **P. Visca****Bianca Colonna** (*Sapienza University of Rome, Italy*)

Hiking the trails of bacterial regulation

Friday, 19th September 2025

(Dept of Law, Via Ostiense 159 - Main Hall and Hall 8)

09.00-11.00 | PLENARY SESSION 5**Virus and host cell interplay**Chairs: **R. De Francesco** and **F. Esposito**

- 09.00 **Urs Greber** (*University of Zurich, Switzerland*)
Dissecting viruses - From stepwise entry to assembly by x-scale imaging
- 09.30 **Lara Manganaro** (*University of Milan, Italy*)
HIV reshapes the transcriptome of CD4+ memory stem cells, silencing cytotoxic programs
- 10.00 **Enzo Tramontano** (*University of Cagliari, Italy*)
A Multi-Omics approach to unveil West Nile Virus interplay with the cellular host
- 10.20 **Marta Giovanetti** (*Università Campus Bio-Medico, Rome, Italy*)
Emerging pathogens: unveiling the impact of climate change on global disease dynamics
- 10.40 **Anna Luganini** (*University of Turin, Italy*)
Calcium-conducting viroporins in viral replication and pathogenesis

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11.00 Coffee Break**11.30-13.30 | PLENARY SESSION 6****Phage Biology: from basics to applications**Chairs: **M. Di Luca** and **A. Danielli**

- 11.30 **Luisa De Sordi** (*Sorbonne Paris, France*)
Of phages and flames: virus-host interactions in inflammatory bowel disease
- 12.00 **Federica Briani** (*University of Milan, Italy*)
Elucidating key steps and players in the adsorption of DEV phage to its *Pseudomonas aeruginosa* host
- 12.25 **Marco Maria D'Andrea** (*University of Rome Tor Vergata, Italy*)
Lytic bacteriophages against *Klebsiella pneumoniae* and their impact on bacterial virulence
- 12.50 **Francesco Santoro** (*University of Siena, Italy*)
Liaisons dangereuses: lysogenic phages, antibiotic resistance, virulence and horizontal gene transfer in *Streptococcus pyogenes*
- 13.10 **Martina Cappelletti** (*University of Bologna, Italy*)
Phages in the dark: exploring the viral diversity and functional potential in subterranean environments

13.30 Lunch and posters viewing (even numbers)**15.00-15.50 | PLENARY LECTURE 3**Chairs: **G. Prosseda** and **L. Leoni****Andres Floto** (*Cambridge University, UK*)

How bacteria evolve from environmental organisms into specialized human pathogens

15.50-16.40 | PLENARY LECTURE 4 (Supported by FEMS)Chairs: **L. Baccigalupi** and **P. Alifano****William Martin** (*Heinrich Heine University, Düsseldorf, Germany*)

Carbon and energy first: Hydrothermal vents and the origin of microbial metabolism

16.40 Coffee Break**17.10 | SHORT PRESENTATION****Matteo Salina** (*Consorzio Italbiotec, Milan, Italy*)**17.20-18.30 | PRIZE PRESENTATIONS****FRANCO TATÒ Prize 2025** (Supported by Consorzio Italbiotec)

Ex aequo:

Claudia Campobasso (*University of Pisa / KU Leuven*)Evolutionary interplay between *Staphylococcus aureus* and phages: strategies to target biofilm and overcome bacterial resistance**Vittorio Giorgio Senatore** (*University of Milano-Bicocca*)

Yeast fermentation and process engineering for the upcycling of PET monomers and agricultural waste

GENPROBIO Prize 2025 (Supported by GenProbio srl)**Lapo Doni** (*University of Genoa*)Global biogeography and ecology of *Vibrio* in a warming planet**SIMGBM Prize 2025 - Best paper****Matteo Cervoni** (*University of Roma Tre*)The diadenosine tetraphosphate hydrolase ApaH contributes to *Pseudomonas aeruginosa* pathogenicity**SIMGBM Prize 2025 - PhD Thesis 2025****Sonia Mirjam Rizzo** (*University of Parma*)

Insights into factors influencing the colonization and composition of the human intestinal microbiota

ADVISE Prize**Giulia Sibille** (*University of Turin*)

Targeting viruses using a novel hDHODH inhibitor

GIUSEPPINA CATTANI Prize**Giovanna Riccardi** (*University of Pavia*)**18.30 Annual assembly of SIMGBM members****20.30 Social dinner****Saturday, 20th September 2025**

(Dept of Law, Via Ostiense 159 - Halls 3, 4 and 9)

9.30-12.30 | PARALLEL SESSIONS - Short talks from selected abstracts**Session A: Microbial genetics and genomics**Chairs: **R. Isticato** and **A. Martorana****Valerio Baldelli** (*University of Milan*)The *Pseudomonas aeruginosa sirB2* gene controls fitness in anaerobic conditions and its inactivation leads to increased virulence and small colony variants emergence via secondary mutations in the *wsp* operon**Matteo Calcagnile** (*University of Salento, Lecce*)Hydroxyl radical generation by PirA connects β -oxidation to oxidative stress: an evolutionarily conserved mechanism?**Marina De Stefano** (*University of Naples*)The *ydaJKLMN* operon mediates the adhesion properties of *Bacillus subtilis* spores**Sarah Hijazi** (*University of Urbino*)Starving the invader: disrupting iron uptake to defeat *Staphylococcus aureus***Giulia Longhi** (*University of Parma*)

The role of teichoic acids in bifidobacteria in mediating the interaction with the human host

Alessia Marotta (*University of Genoa*)*memod-s*: a standardised workflow to explore and analyse prokaryotic methylation patterns for Nanopore sequencing data**Diego Marco Minore** (*University of Milan*)

Exploring bacterial evolution across the genome architecture landscape

Riccardo Polani (*Sapienza University of Rome*)Conjugative CRISPR-Cas vector to cure KPC-encoding plasmids in *Klebsiella pneumoniae*

Cinzia Spagnoli (*University of Roma Tre*)

Molecular mechanisms implicated in the resuscitation of *Acinetobacter baumannii* Viable But Non-Culturable (VBNC) cells induced by desiccation

Davide Sposato (*University of Roma Tre*)

Role of DedA proteins in *Pseudomonas aeruginosa*

Giovanni Stelitano (*University of Pavia*)

Repurposing FDA approved drugs to disrupt *Mycobacterium abscessus* iron acquisition pathway by targeting the salicylate synthase Mab-SaS

Mariana Tirziu (*University of Siena*)

Genome analysis and mobilome characterization of *Trichomonas vaginalis*-associated *Mycoplasma hominis* isolates through whole genome sequencing

Session B: Environmental and industrial microbiology

chairs: **E. Bona** and **T. Rinaldi**

Silvia La Scala (*University of Palermo*)

Plant growth-promoting Actinomycetota modulate bioactive metabolites profiles in mediterranean plants

Daniela Sateriale (*University of Sannio, Benevento*)

Plant growth-promoting rhizobacterial consortium from the Rhizosphere of *Vitis vinifera* cv. Falanghina: a terroir-oriented approach to sustainable agricultural biocontrol

Eleonora Metta (*University of Cagliari*)

Preliminary characterization of the microbial community living in a multi-pond solar saltern located in south Sardinia

Jacopo Brusca (*University Ca' Foscari of Venezia*)

Climate change impact on soil microbial communities of the West Antarctic Peninsula

Alessandro Russo (*University of Florence*)

Bioplastic in sandy beaches: the involvement of gut bacterial communities from supralittoral amphipods in polymers degradation

Flavia Cannizzaro (*University of Palermo*)

Extracellular membrane vesicles from *Streptomyces violaceoruber*: a promising natural strategy for enhancing plant growth and health

Marta Nerini (*University of Florence*)

Developing a drone-based system for rapid bioaerosol sampling

Silvia Abbà (*University of Turin*)

Dissecting molecular, physical, and chemical factors of wasps' gut shaping *Saccharomyces cerevisiae* survival

Melinda Mandaresu (*University of Cagliari*)

Study of microbial diversity associated to *Helichrysum microphyllum* subsp. *tyrrhenicum*, a metallophyte endemic of Sardinian mining areas

Filippo Pasquale Riggio (*University of Roma Tre*)

Spatiotemporal variations of the bacterial communities colonizing five pyroclastic caves located in the Rome metropolitan area

Session C: Interactions between microbes/viruses and their hosts

chairs: **F. Iannelli** and **R. Proveddi**

Alessia Avesani (*University of Genoa*)

First insights into bacterial and microalgal endosymbiont communities of various coral morphotypes from Maldives

Andrea Bonacorsi (*University of Pisa*)

Pisa 4: *in vitro* characterization and genetic engineering of a novel mycobacteriophage for therapeutic use

Elena Capuzzo (*Institut Pasteur, Paris, France*)

Control of *Staphylococcus aureus* infection in burn wounds by physical plasma

Antonella Congiargiu (*University of Sassari*)

Unraveling the microbial and immune landscape of Prostate Cancer

Umberto Gabriele D'Oria (*University of Trento*)

Investigating Flavivirus tropism and entry mechanisms in human cells

Stefano Nenciarini (*University of Florence*)

Isolation source shapes the cargo of *Saccharomyces cerevisiae* extracellular vesicles

Tommaso Olimpieri (*University of Roma Tor Vergata*)

Enhanced clearance of phage-resistant *Klebsiella pneumoniae* strains by the innate immune system

Silvia Caterina Resta (*University of Salento, Lecce*)

Emricasan (IDN-6556) inhibition of caspase-3/gasdermin-E pyroptosis pathway during *Neisseria meningitidis* infection could block bacterial epithelial barrier crossing

Alessandro Stamilla (*University of Pavia*)

Granuloma-like structures in tuberculosis: a hybrid experimental and modeling approach for drug testing

Debora Stelitano (*Telethon Institute of Genetics and Medicine, Pozzuoli*)

Unveiling the role of peroxisomes in SARS-CoV-2 Pathogenesis

Chiara Tarracchini (*University of Parma*)

Biogeography of the infant gut microbiota and identification of key microbial members

Rita Triocco (*Sapienza University of Rome*)

Loss of FadD reduces *Shigella flexneri* invasiveness by impairing of the Type III Secretion System

ABSTRACTS

OPENING LECTURE

Antibiotic Persistence: Exploring Mechanisms and Evolutionary Pathways

J. Michiels

Centre of Microbial and Plant Genetics, KU Leuven, Belgium

Center for Microbiology, VIB-KU Leuven, Belgium

jan.michiels@kuleuven.be | www.michielslab.org | https://michielslab.sites.vib.be/en

Persister cells constitute a subset of phenotypic variants in bacterial isogenic populations that exhibit transient tolerance to lethal antibiotic therapy. They have been associated with recurrent infections and failure of antibiotic treatment. I will present our contributions to the understanding of how persisters develop and recover following antibiotic treatment, exploring both mechanistic and evolutionary aspects, with a particular focus on the relationship to antibiotic resistance. Moreover, I will discuss the role of protein aggregation and aggregate structure in the development of dormancy.

PLENARY LECTURES

Architecture, assembly and mechanism of action of an antibacterial speargun: the type VI secretion system

E. Cascales

Laboratory of Engineering of Macromolecular Systems (LISM), Mediterranean Institute of Microbiology (IMM), CNRS, Aix-Marseille University, France

The type VI secretion system (T6SS) is a nanoweapon used by bacteria to target both prokaryotic and host cells, therefore participating to interbacterial competition and pathogenesis. The T6SS uses a contractile mechanism to deliver protein effectors. It is comprised of an envelope-spanning complex anchoring a cytoplasmic tubular edifice. This tubular structure is evolutionarily, functionally and structurally related to the tail of contractile phages. It is comprised of a needle, composed of an inner tube tipped by a spike complex, engulfed within a sheath-like structure. This structure assembles onto a platform called «baseplate» that is recruited and anchored to the membrane complex. The T6SS functions as a nano-speargun: upon sheath contraction, the needle is propelled into the target cell such as an arrow, allowing the delivery of highly potent toxins. I will present the general architecture, the biogenesis and the mode of action of this fascinating secretion machine, and will discuss the activity and mechanism of delivery of selected toxins.

Hiking the trail of bacterial regulation

B. Colonna

Dipartimento di Biologia e Biotechnologie "Charles Darwin", Istituto Pasteur Italia, Sapienza Università di Roma, Italy

Bacterial pathogens often must survive within fundamentally diverse habitats. Their ability to dynamically adapt to their surroundings depends on their capacity to sense environmental variations and respond appropriately, which frequently involves drastic changes in the cell's transcriptional program. Swiftly orchestrating gene expression requires the bacterium to invest

not only in numerous gene functions that facilitate adaptation to different milieus, but also in regulatory functions that allow it to modulate its response in a coordinate manner to shifting environmental conditions. Therefore, pathogens represent a fantastic model for the study of gene regulation, as the complexity of their mechanisms can be seen as an evolutionary response to the challenge of surviving in changing environments. *Shigella* is a highly adapted human pathogen, mainly found in the developing world and causing a severe enteric syndrome. As in other life-threatening human pathogens, in *Shigella* the regulation of invasive genes, mainly located on large virulence plasmid, occurs at multiple levels and is modulated by host-derived environmental stimuli.

Like a long and fascinating trail revealing new landscapes at every step, our group explored the strategies used by *Shigella* to coordinately activate the invasive process once inside the host. Through our studies analyzing the interplay, either synergistic or antagonist, among nucleoid associated proteins, sRNA, global and specific regulators, two component systems and intrinsic features of the DNA we have contribute to decipher how *Shigella* has become a successful pathogen able to prevent wasteful expression of virulence factors while ensuring strong appropriate activation when this is required.

How bacteria evolve from environmental organisms into specialized human pathogens

A. Floto

Department of Medicine, University of Cambridge

I will discuss our recent work on how environmental bacteria evolve to become specialised human lung pathogens using two example species: *Mycobacterium abscessus* and *Pseudomonas aeruginosa*.

M. abscessus is an intrinsically multidrug-resistant species of nontuberculous mycobacteria (NTM) that has recently emerged as a major threat to individuals with Cystic Fibrosis (CF) and other chronic lung conditions, including non-CF bronchiectasis. In CF, *M. abscessus* drives accelerated inflammatory lung damage, is frequently impossible to treat, and usually prevents safe lung transplantation. Infection rates are increasing globally, which we have shown are driven (at least in part) by indirect person-to-person transmission of *M. abscessus*, probably through the generation of long-lived infectious aerosols and via fomite spread. Although *M. abscessus* was originally thought to be independently acquired from the environment, we have shown that over 70% of infections in CF patients are caused by genetically clustered (and thus transmitted) isolates, of which the majority are from three dominant circulating clones (DCCs) that synchronously emerged in the 1960s and have spread globally in mixed transmission networks involving both CF patients and (based on the mutational signatures in the bacteria) smokers. Clustered isolates are more virulent (when tested *in vitro* and *in vivo*) and result in worse clinical outcomes, suggesting that they are evolving from environmental saprophytes into professional lung pathogens in a similar way to ancestral *M. tuberculosis* over 6000 years ago.

M. abscessus has provided a unique opportunity to define the critical (and generalisable) steps involved in pathogenic evolution of mycobacteria involving: (i) saltational evolution of virulence in specific environmental clones driven by horizontal gene transfer; (ii) allopatric, within-host adaptation during chronic infection increasing survival within macrophages; (iii) constrained pathogenic evolution while transmission is via environmental intermediaries; and finally (iv) accelerated evolution into an obligate lung pathogen. I will describe our work on bacterial evolution, host susceptibility, and how these finding might be generalisable across bacterial species, particularly *Pseudomonas aeruginosa*.

References

- ¹ Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, Verma D, Emily Hill E, Drijkoningen J, Gilligan P, Esther CR, Noone PG, Giddings O, Bell SC, Thomson R, Wainwright CE, Coulter C, Pandey S, Wood ME, Stockwell RE, Ramsay KA, Sherrard LJ, Kidd TJ, Jabbour N, Johnson GR, Knibbs LD, Morawska L, Sly PD, Jones A, Bilton D, Laurenson I, Ruddy M, Bourke S, Bowler ICJW, Chapman SJ, Clayton A, Cullen M, Daniels T, Dempsey O, Denton M, Desai M, Drew RJ, Edenborough F, Evans J, Folb J, Humphrey H, Isalska B, Jensen-Fangel S, Jönsson B, Jones AM, Katzenstein TL, Lillebaek T, MacGregor G, Mayell S, Millar M, Modha D, Nash EF, O'Brien C, O'Brien D, Ohri C, Pao CS, Peckham D, Perrin F, Perry A, Pressler T, Prtak L, Qvist T, Robb A, Rodgers H, Schaffer K, Shafi N, van Ingen J, Walshaw M, Watson D, West N, Whitehouse J, Haworth CS, Harris SR, Ordway D, Parkhill J, Floto RA (2016). Emergence and spread of a human- transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 354: 751-757.
- ² Ruis C, Bryant JM, Bell S, Thomson R, Davidson RM, Hasan NH, van Ingen J, Strong M, Floto RA* (joint corresponding), Parkhill J*. Dissemination of *Mycobacterium abscessus* via global transmission networks (2021). *Nature Microbiology* doi.org/10.1038/s41564-021-00963-3
- ³ Bryant JM, Brown KP, Burbaud S, Everall I, Belardinelli JM, Rodriguez-Rincon D, Grogono DM, Peterson CM, Verma D, Evans IE, Ruis C, Weimann A, Arora D, Malhotra S, Bannerman B, Passemar C, Templeton K, MacGregor G, Jiwa K, Fisher AJ, Blundell TL, Ordway DJ, Jackson M, Parkhill J, Floto RA. Stepwise pathogenic evolution of *Mycobacterium abscessus*. *Science*. 2021 Apr 30;372(6541): eabb8699. doi: 10.1126/science.abb8699. PMID: 33926925.
- ⁴ Boeck L, Burbaud S, Skwark M, Pearson WH, Sangen J, Weimann A, Everall I, Bryant JM, Malhotra S, Bannerman BP, Kierdorf K, Blundell TL, Dionne M, Parkhill J, Floto RA (2022) The pathobiology of *Mycobacterium abscessus* revealed through phenogenomic analysis. *Nature Microbiology* 7, 1431–1441
- ⁵ Weimann A, Dinan AM, Ruis C, Bernut A, Pont S, Brown K, Ryan J, Santos L, Ellison L, Ukor E, Pandurangan AP, Krokowski S, Blundell TL, Welch M, Blane B, Judge K, Bousfield R, Brown N, Bryant JM, Kukavica-Ibrulj I, Rampioni G, Leoni L, Harrison PT, Peacock SJ, Thomson NR, Gauthier J, Fothergill JL, Levesque RC, Parkhill J, Floto RA. Evolution and host-specific adaptation of *Pseudomonas aeruginosa*. *Science*. 2024 Jul 5;385(6704):eadi0908. doi: 10.1126/science.adi0908. Epub 2024 Jul 5. PMID: 38963857.

Carbon and energy first: Hydrothermal vents and the origin of microbial metabolism

W.F. Martin

University of Düsseldorf, Germany

Life is a chemical reaction. All cells require carbon, energy, and electrons for growth. One pathway serves all three needs simultaneously. In the acetyl-CoA pathway, H₂ reduces CO₂ to pyruvate for carbon supply, while methane (archaea) or acetate (bacteria) synthesis are coupled to ion pumping and energy conservation via the rotor-stator ATP synthase. Simplicity and thermodynamics implicate the acetyl-CoA pathway as the most ancient route of carbon assimilation. Fe, Co, and Ni in the active sites of its enzymes are relics of origins. Using Fe⁰ or Ni⁰ as catalysts, aqueous H₂ and CO₂ react specifically to formate, acetate, methane, methanol, and pyruvate overnight at 100 °C in the laboratory. Solid state Ni⁰ alone can replace the function of 140 enzymes required to convert H₂ and CO₂ to pyruvate and methane in cells, Ni⁰ can also catalyze reductive aminations of 2-oxoacids, while Fe⁰ reduces the 4Fe4S clusters of ferredoxin—an evolutionary precursor of flavin-based electron bifurcation. The alkaline conditions (pH 8-11) of serpentinizing (H₂-producing) hydrothermal systems generate very reducing conditions (E₀' up to -900 mV), depositing Fe⁰, Co⁰, and Ni⁰ as metals. These are precisely the metals that i) reduce CO₂ with H₂ in the laboratory and ii) are present at the active sites of enzymes in the acetyl-CoA pathway. Organic synthesis from H₂ and CO₂ on solid state metals unites hydrothermal environments, ancient microbial metabolism, and inorganic catalysts, converging on the origin of metabolism.

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Plenary Session 1

Planetary microbiology: the role of microorganisms in a changing earth

Microbiology of volcanic gases and the metabolism of one carbon compounds

M. Hernández

University of East Anglia, UEA

Utilisation of trace gases such as carbon monoxide (CO), hydrogen, and methane by bacteria in volcanic soils has been shown to be important in early soil development and bacterial community establishment. In these extreme environments, the enzyme carbon monoxide dehydrogenase oxidises CO to carbon dioxide and energy. In our work, we investigate CO metabolism in volcanic soils from these environments. Metagenomics and enrichment strategies targeting carboxydovores - bacteria capable of oxidising low-to-ambient CO concentrations - were employed to isolate novel strains. From Calbuco Volcano, Chile, two carboxydovores, *Cupriavidus ulmosensis* CV2^T and *Paraburkholderia terrae* COX, were identified. Whole-genome sequencing and CO consumption assays revealed distinct mechanisms regulating CO metabolism during transitions from heterotrophy to starvation, providing insight into microbial survival in extreme environments. Both strains contain *coxMSL* genes, which encode CO dehydrogenase responsible for CO oxidation, along with uncharacterised accessory genes. Our work also explores the active microbes consuming CO in volcanic soils using stable isotope probing (SIP) with ¹³CO as the substrate (with and without the presence of hydrogen) showing that Pseudomonadota are highly abundant. Our findings emphasise the significance of CO metabolism in early soil formation and bacterial community establishment, presenting novel model strains to further investigate microbial adaptations in volcanic ecosystems.

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Community network models to reveal marine plankton systems ecology and evolution

S. Chaffron^{1,2}

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Marine plankton form complex communities of interacting organisms at the base of the food web, which sustain oceanic biogeochemical cycles and regulate climate. Identifying the mechanisms controlling their assembly and activities is a major challenge in microbial ecology. Though global surveys are starting to reveal ecological drivers underlying planktonic community structure and predicted climate change responses, it is unclear how community-scale species interactions are constrained, and how they will be affected by climate change. By leveraging Tara Oceans metagenomics data, plankton community network models can be integrated with niche modelling to reveal biome-specific plankton community responses to environmental change, and forecast most affected lineages within each community. To go beyond statistical models, genome-resolved community networks enable to model and predict metabolic cross-feedings within prokaryotic assemblages. These mechanistic models allow to predict potential interactions within predicted communities and pinpoint specific metabolic cross-feedings shaping plankton microbial communities. Combining statistical ecological models with mechanistic metabolic models provides a useful framework to assess community structure and organismal interactions, to reveal central mechanisms shaping natural microbial community ecology and evolution in our changing ocean.

Geosphere-Biosphere coevolution: the role of trace metal availability in the evolution of biogeochemistry

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Life and our planet have significantly coevolved for the past 4 billion years. A large swot of this interaction is mediated by the capacity of life to mediate redox reactions at the interface between the geosphere and the biosphere. In order to control redox chemistry, life relies on diverse trace elements used as cofactors that need to be tuned to the midpoint potential of the substrate to function properly. Metals such as Fe, Co, Ni, Zn, Mo, W, V, and Cu are used in these proteins and constitute essential micronutrients for microbial growth. Given that the availability of many of the essential trace elements has changed as a function of changing planetary conditions in deep time, unveiling the link between trace element availability and functional diversity might shed light on the evolution of metabolism and the emergence of biogeochemistry. Here, we will present recent data from field and laboratory experiments elucidating the impact of trace element availability on microbial functional diversity, showing that trace elements can be used to manipulate microbial metabolisms controlling the shift between diverse electron acceptors. We further show that this mechanism is widespread in the tree of life and that the scarcity of key trace elements imposes additional metabolic costs on the fitness of microorganisms. Together, our results suggest a strong feedback between changing planetary redox conditions and possible utilization of new redox couples, highlighting new feedbacks at the interface between geosphere and biosphere.

The role of the soil microbiota in mitigating desertification in Southern Europe

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Soil is a vital nonrenewable resource that allows life on earth thanks to its countless ecosystem services. Despite having a central role in several SDG, sustainable soil management and soil security continue to be scarcely considered in politics and society, and also poorly addressed by scientists.

Intensive anthropic land uses are depauperating soil, while drought and extreme weather events contribute to its degradation, especially in semi-arid Mediterranean regions where erosion, compaction, salinization, reduction of organic matter and biodiversity loss, lead to desertification, with negative consequences for the environment and food supply.

The soil microbiota is recognized as a key player in the functioning and productivity of (agro) ecosystems and experimental evidence is accumulating that preventing desertification and improving soil health includes boosting the soil microbiome. Nevertheless, we still lack basic

knowledge of the drivers of soil microbial diversity and functions and how soil management can modulate microbial functions in degraded/desertified soils.

Microbial diversity of southern European soils at high desertification risk, was monitored within the project LIFE Desert Adapt (<http://www.desert-adapt.it/index.php/en/>), to shed light on the relationships between soil biodiversity, pedoclimatic conditions and land use. The final aims are *i)* to identify the main drivers of microbial soil functions that contribute to mitigate the effects of climate change and improve soil fertility and *ii)* to create the microbial diversity baseline useful for monitoring the effects of climate change, land use, and adaptation measures implemented in the agricultural fields, to support technical and political decisions.

Plenary Session 2

Multidisciplinary approaches to tackle antibiotic resistance

Unlocking new drug modes of action by multidimensional high- throughput metabolic profiling

Dr. M. Zampieri

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Understanding a small molecule's Mode of Action is essential to guide lead compound selection, optimization and clinical development. I will present a multiparametric approach that by linking genetic to drug-induced changes in nearly a thousand metabolites, enables high-throughput functional annotation of compound libraries, and discuss how the systematic molecular profiling of small molecules interference with metabolism can change how we search for new and unconventional antibacterial agents.

Genetics and evolution of polymyxin resistance in *Pseudomonas aeruginosa*

F. Imperi

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Polymyxin antibiotics represent one of the last-resort treatment options for infections caused by multidrug-resistant Gram-negative pathogens. However, reports of polymyxin resistance are increasing worldwide. Understanding how resistance to polymyxins emerges and influences bacterial fitness, as well as elucidating its genetic and molecular basis, is important for developing effective surveillance or therapeutic strategies to preserve the clinical efficacy of these antibiotics. In this talk, I will discuss the polymyxin resistance mechanisms of the human pathogen *Pseudomonas aeruginosa*, their relative contributions to resistance, and the molecular, physiological, and evolutionary factors that have likely shaped their success as resistance determinants.

Microbial interactions and antibiotic resistance

M. Fondi

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Up to now, antibiotic resistance (AR) has been mostly studied from the point of view of single isolates and how different drugs could affect their phenotype and their genotype. However, in recent years, the study of microbial communities and how their ecological relationships could alter the efficacy of antibiotics has gained ground, leading to the hypothesis that inter-species interactions may have a major role in affecting the survival of community members during antibiotic exposure. Life within a community can sometimes favor sensitive strains, allowing them to grow in concentrations higher than their minimum for inhibition in pure culture. Such microorganisms, for example, can be physically protected by the biofilm layer produced by the other community members or by the active degradation of the antibiotic molecules by their neighbors. Similarly, the evolution and spreading of AR can also be influenced by the community microbes live in. Although these findings lead to the worrisome outcome that interactions within a microbiota can significantly affect the outcome of treatment regimens, their quantitative interpretation has been greatly overlooked up to now, due to the complexity of real microbial communities and of their surroundings. Theoretical models, however, allow dealing with the complexity of such biological systems and may indicate new possible directions of general improvement in antibiotic therapies. Here, we will provide an overview of microbiota-aware studies of AR evolution and spreading, together with theoretical considerations and simulations on how the structure of microbial communities can impact AR and AR-related phenotypes (e.g. persistence).

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Novel in vitro tools to inform pre-clinical and clinical progression of TB combination therapy

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Novel tuberculosis (TB) combination therapies have been recommended by the WHO. Understanding how to efficiently develop such regimens from inception remains, however, a paramount challenge.

The main limitation of traditional in vitro methods used to develop drug combinations, such as checkerboard assays (CBA), is the use of growth inhibition as a metric of drug activity (based on MIC determinations). This requires secondary validation assays typically performed by the gold-standard in vitro proxy time-kill assay (TKA). Unlike CBA, TKA rely on a bactericidal parameter (Log₁₀CFU/mL) and is basis of pharmacometrics modelling of antimicrobial drug action. However, compared to the fixed endpoint for CBA, TKA rely on CFU enumeration at different time points, which requires large culture volumes, long readout times and has a limited throughput capacity. This creates a barrier to validate interactions of more than 3 drugs per experiment. In addition, TKA rely on static drug concentrations added at the beginning of the experiment, which can undergo degradation processes, thus generating uncertainty on the actual bacterial exposure to the drug. To overcome these limitations, we have implemented two novel methodologies named OPTIKA (Optimized Time Kill Assays) and the Hollow Fiber System for Tuberculosis (HFS-TB). In this talk, I will describe how OPTIKA and HFS-TB, coupled to pharmacometrics modelling, can play a critical role in the preclinical and clinical development of novel anti-TB combinations. This work has received support from the Innovative Medicines Initiatives 2 Joint Undertaking (grant No 853989).

Plenary Session 3

New frontiers in human and animal microbiome research

Development of the gut microbiota-immune-brain axis in extremely premature infants

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Extremely premature infants, born before the 28th week of gestation with <1 kg body weight, are at high risk of experiencing acute and long-term adverse outcomes. Brain development occurs in these patients in an extrauterine environment and in the presence of myriad environmental antigens including colonizing microorganisms, which may affect neurodevelopment and long-term cognitive and behavioral performance.

Here, I will describe the ecological processes governing the assembly and succession of the gut microbiota of extremely premature infants. We evaluated microbial community load, composition, development, and resistance and resilience of the microbiota to perturbations. Additionally, we identified bacteria and metabolites whose abundance was significantly associated with a subsequent diagnosis of brain injury, as determined by cranial magnetic resonance imaging (cMRI) at discharge. Using long-read metagenomics, we found that although there were no differences in alpha diversity associated with brain injury, there were large-scale changes in the community members participating in major biochemical pathways. Microbiotas in patients with brain injury were enriched in species with elevated antibiotic resistance gene repertoires and inflammation-associated genomic features. Additionally, an altered microbiota was associated with delayed neurodevelopment and altered immunological properties.

These results indicate that the microbiota is linked to clinical outcome in extremely premature infants and that microbiome-targeted interventions may be a promising strategy to promote development of a beneficial gut microbiome.

Gut microbiota drives colon cancer risk associated with diet: a comparative analysis of meat-based and pesco-vegetarian diets

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Colorectal cancer (CRC) risk is strongly influenced by dietary habits, with red and processed meat increasing risk, while fiber-rich diets are considered protective. Diet also shapes the gut microbiota, but the combined role of diet, microbiota, and metabolic profiles in CRC risk remains incompletely understood. To explore this interaction, *Apc*-mutated PIRC rats and azoxymethane (AOM)-treated rats were fed a high-risk meat-based diet (MBD), the same diet with α -tocopherol (MBDT), a low-risk pesco-vegetarian diet (PVD), or a control diet. Faecal microbiota transplantation (FMT) from PIRC rats was performed into germ-free AOM-treated rats fed a standard diet for three months.

In both models, PVD-fed rats showed significantly lower tumorigenesis than MBD-fed rats, consistent with several CRC biomarkers. Faecal microbiota and metabolite profiles varied clearly by diet. Notably, germ-free rats receiving MBD-derived microbiota developed more preneoplastic lesions and showed bacterial and metabolic signatures reflective of the donor diet. PVD was associated with nine protective taxa, mainly from Lachnospiraceae and Prevotellaceae, with four shared across all models. These correlated inversely with nonconjugated bile acids, suggesting a potential protective mechanism.

These results highlight the protective effects of PVD and reaffirm the carcinogenic potential of MBD. In germ-free rats, FMT induced changes mimicking those of the donor diet, including increased lesions in MBD recipients. Crucially, this study is the first to demonstrate that diet-associated CRC risk can be transmitted via faeces, establishing the gut microbiota as a key determinant of diet-related cancer susceptibility.

Bifidobacteria as a model microbial group of the human gut Microbiome

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Bifidobacteria are among the earliest colonizers of the human gastrointestinal tract and represent a key microbial group not only in the infant gut microbiome but also throughout adulthood and old age. These Gram-positive, anaerobic bacteria play a crucial role in maintaining gut homeostasis, modulating immune responses, and contributing to the metabolism of complex carbohydrates through their unique enzymatic capabilities. Due to their health-promoting properties and prevalence in the gut, bifidobacteria are often used as probiotics and serve as a model for studying host-microbe interactions. Recent advances in genomic and metagenomic technologies have enabled more profound insights into their genetic diversity, adaptation mechanisms, and ecological significance. Studies have shown that bifidobacteria can influence the composition and function of the broader microbial community and interact closely with the host through the production of short-chain fatty acids and other metabolites. Given their role within the gut ecosystem, bifidobacteria provide a valuable model for understanding the dynamics of the human gut microbiome and for developing microbiome-based therapeutic strategies. Ongoing research continues to explore their potential in promoting health and preventing or managing gastrointestinal and systemic diseases.

Polyphenol-rich by-products to modulate the activity of rumen microbiota

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The rumen is the largest stomach in ruminants and hosts a complex microbial community which main role is to degrade the fibers ingested by the animal. Sugars derived from fibers degradation are then fermented to volatile fatty acids (the primary energy source for the animal), CO₂ and CH₄. Despite a core rumen microbiome exists, the composition of the microbial community in the rumen can be influenced by several factors, such as diet. Polyphenols are plant metabolites with a phenolic group that can be present in ruminants' feed, or that can be added to the feed (e.g., by inclusion of agro-industrial by-products). Polyphenols can modulate the microbial activity by decreasing enteric CH₄ production, with a positive impact on the environmental sustainability of animal productions. Furthermore, the presence of polyphenols in ruminants' diet can modulate the fatty acids (FAs) biohydrogenation (i.e., the progressive isomerization and saturation

of dietary fatty acids in the rumen), a microbiological process that influences the FAs profile and consequently the quality of animal products. Despite the effect of polyphenols on rumen microbiome is different according to animal species, polyphenol source and level of inclusion in the diet, there is general evidence that the content of saturated FAs decreases by feeding polyphenol-rich diets, while the content of conjugated linoleic acid isomers (i.e., FAs that can improve nutritional value) increases. The inclusion of polyphenol-rich by-products in ruminants' diet is thus a valuable strategy to improve the quality of animal products and to decrease the environmental impact of livestock productions.

***In vitro* models as cutting-edge tools for studying the gut microbiota: from static bioreactors to pocket dynamic platforms**

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The gut microbiota plays crucial roles in intestinal and systemic host physiology. *In vitro* models for culturing the gut microbiota recently emerged as powerful tools complementing clinical trials and animal studies to unravel microbiota-associated diseases and potential preventive/therapeutic strategies. Here, we provide an overview of the current *in vitro* systems used for culturing the human gut microbiota, tracing their evolution from conventional batch bioreactors to cutting-edge microfluidic devices. Among these, we focus on the two scaffold-based *in vitro* models we developed, named 3DM-IVM and MICRO-B. Both models are based on the use of electrospun gelatin membranes as scaffolds for microbial adhesion and growth during the *in vitro* culture. While 3DM-IVM is a static multi-well system useful to largely screen effects of compounds, pathogens, and probiotics on the gut microbiota, MICRO-B is a dynamic millifluidic platform implemented with flow and peristalsis, which well reproduces the intestinal environment. 3DM-IVM was used to test the role of intestinal mucus, *Bacillus cereus* (as an enteropathogen), and *Bacillus clausii* (as a probiotic) in modulating the microbial composition *in vitro*, providing findings that potentially can be translated *in vivo*. MICRO-B is currently under validation, but its physiological relevance is very promising. The application of *in vitro* models in pre-clinical settings could represent the bridge to fill the gap of knowledge on the gut microbiota, which nowadays is still largely unknown.

Plenary Session 4

“Survive, escape, and kill”: mechanisms of host-pathogen interactions

Host directed therapies for mycobacterial infections

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The emergence and spread of drug-resistant strains of *Mycobacterium tuberculosis* is worsening the global threat of tuberculosis (TB). There is a need and urgency for the development of new treatments for TB, for the management of drug-resistant TB (MDR-TB) and for improved regimens against drug-susceptible TB, with the goal of reducing toxicity and length of therapy that will

boost patient compliance. Similarly, the last decade has seen an increase in the incidence of mycobacterial diseases, often caused by species with significant pathogenic potential and intrinsically resistant to antibiotics, as in the cases of *Mycobacterium abscessus* and *M. avium*. The paucity of new drugs is a major obstacle to design new and effective regimens against mycobacterial infections. Recently, host-directed therapies (HDTs) are emerging as a promising area of research and are opening new avenues to fight TB. Starting from a better understanding of TB pathogenesis and most importantly from the empirical observation that in most cases the host immune responses can control mycobacteria preventing any form of sign or symptom of disease, it is possible to act on the host to contain mycobacterial replication or curb immunopathological responses. Repurposing new drugs, targeting specific host pathways may pave the way for the design of new regimens that may include HDTs as adjunct therapy for TB and mycobacterial diseases.

Not macrophages but sinusoids capture bacteria in the human spleen

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Human tissue macrophages, such as alveolar macrophages, hepatic Kupffer cells, and meningeal macrophages, express CD14, CD68, CD163, CD169, and CD206. Among these markers, the CD206 mannose receptor plays a crucial role as an endocytic receptor that captures bacteria via their polysaccharide capsule. In the human spleen, CD163 is associated with red pulp macrophages, CD169 with perifollicular arteriolar sheath macrophages, and CD206 with sinusoidal lining cells. To investigate pneumococcal clearance, we infected whole human spleens during ex vivo organ perfusion. Pneumococci of various capsular serotypes were cleared with similar efficiency, removing approximately ninety percent of the bacteria from the challenge dose within an hour. Microscopic examination of tissue biopsies taken over time showed bacteria present on sinusoidal lining cells or inside macrophages. To assess the role of the mannose receptor on human sinusoidal spleen cells, we added mannose to the perfusion fluid. This completely halted pneumococcal clearance and subsequent phagocytosis. Our findings suggest that, unlike in the mouse spleen, effective killing of pneumococci in the human spleen relies entirely on their initial capture by sinusoidal lining cells.

LuxR solos represent a major family of cell-cell signaling regulators in the plant microbiome

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LuxR solos represent a widespread and functionally diverse family of cell-cell signaling regulators, particularly enriched in plant-associated bacteria. Unlike quorum sensing canonical LuxR regulators that function in tandem with LuxI-produced acyl-homoserine lactone (AHL) signals, LuxR solos operate without a nearby LuxI synthase and detect a broader range of endogenous, exogenous or host-derived signals. Recent genomic analyses have revealed that a substantial proportion of LuxR solos are genetically linked to uncharacterized biosynthetic gene clusters (BGCs), suggesting LuxR solos respond to non-AHL metabolites produced by adjacent BGCs. This emerging model points to the existence of highly specialized communication systems within plant microbiomes, where multiple distinct chemical languages operate in parallel, involved in

the regulation of microbial behavior, niche adaptation, and plant interaction. These systems are especially prevalent in genera such as *Pseudomonas*, *Rhizobium*, and *Burkholderia*, which are strongly associated with root, leaf, and rhizosphere habitats. Understanding the molecular diversity, structural specificity, and evolutionary origin of LuxR solos, particularly in relation to BGCs, offers a possibility for discovering novel cell-cell signals as well as bioactive compounds with potential agricultural applications.

Fatal Attraction: role of adhesins in the virulence of the opportunistic pathogen *Candida parapsilosis*

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Adhesion is the first and most crucial step in the establishment of fungal infections, enabling *Candida parapsilosis* to colonize both host tissues and medical devices. As an emerging opportunistic pathogen, *C. parapsilosis* relies on a family of cell wall glycoproteins known as agglutinin-like sequence (Als) proteins to mediate this adhesion. Our work expands the current understanding of this protein family by functionally characterizing all five *C. parapsilosis* ALS genes (*CpALS4770*, *CpALS4780*, *CpALS4790*, *CpALS0660*, and *CpALS4800*). Using targeted gene deletion or gene editing strategies, we show that disruption of *CpALS4770* or *CpALS4790* significantly impairs adhesion to human buccal epithelial cells, while the *CpALS4770-CpALS4780* double mutant paradoxically enhances adhesion and aggregation. Notably, deletion of *CpALS4800* results in a >60% reduction in adhesion and attenuated virulence in a murine urinary tract model, reinforcing its critical role. Similar in vivo attenuation was observed for *CpALS4790* and *CpALS0660* mutants in a model of vaginal candidiasis, despite their near-normal in vitro phenotypes. Importantly, these findings highlight specific ALS proteins as attractive targets for antifungal therapies aimed at preventing colonization and biofilm formation. By disrupting adhesion mechanisms, the risk of persistent infections and device-associated mycoses could be potentially reduced. This work lays the foundation for translational strategies focused on adhesion-blocking agents targeting key ALS adhesins in *C. parapsilosis*.

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Plenary Session 5

Virus and host cell interplay

Dissecting viruses - From stepwise entry to assembly by x-scale imaging

U.F. Greber

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Adenoviruses (AdV) first isolated in 1953 are widespread pathogens in vertebrates, and feature >120 genotypes in humans. AdV infect the respiratory and gastrointestinal tracts, eyes, heart, liver, and kidney, persist in immune cells, and elicit life-threatening disease in immunosuppressed individuals¹. The clinical importance of AdVs and their broad application as gene vectors have prioritized mechanistic studies^{2,3}. Taking a combined chemical, cell biological and systems approach through click-chemistry, cryo-electron tomography and mass-spectrometry I will discuss new insight into viral DNA (vDNA) uncoating at the nuclear pore complex and vDNA delivery into the nucleus. Alone, the nucleus is also the site for vDNA replication and progeny formation. I will discuss an emerging model for concurrent vDNA packaging and capsid formation⁴⁻⁶. The results

lay a foundation for systematically exploring host and viral factors in virion morphogenesis, and addressing the pathways in lytic and nonlytic virus cell egress⁷, major blind spots in adenovirology^{3,8}.

References

- ¹Lion T. 2019. *FEBS Lett* 593: 3571-82
²Greber UF, Flatt JW. 2019. *Annu Rev Virol* 6: 177-97
³Hearing P. 2021. *Fields Virology: DNA viruses*, pp. 98-128
⁴Charman M et al. 2023. *Nature* 616: 332-8
⁵Gomez-Gonzalez A et al. 2024. *Sci Adv* 10: eadq7483
⁶Greber UF. 2025. *J Virol*: e0047125
⁷Georgi F et al. 2020. *Antimicrob Agents Chemother* 64(9):e01002-20
⁸Charman M, Weitzman MD. 2025. *J Virol*: e0018025

HIV reshapes the transcriptome of CD4+ memory stem cells, silencing cytotoxic programs

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Despite effective antiretroviral therapy (ART), HIV persists in latent CD4+ T cells, hindering eradication. Additionally, some individuals experience ongoing immune dysfunction, T cell exhaustion, and chronic inflammation despite undetectable viremia.

This study examines how HIV affects CD4+ T memory stem cells (TSCM), a long-lived, self-renewing subset that may serve as an HIV reservoir and support immune homeostasis.

Using bulk RNA sequencing (RNA-Seq), we investigated how HIV infection alters the transcriptomic landscape of TSCM. CD4+ T cells from five donors were infected with a GFP-reporter HIV virus, TSCM were subsequently sorted into GFP+ and GFP- populations for analysis.

An interferon-stimulated gene signature was strongly upregulated in both GFP+ and GFP- cells compared to controls, but was absent in the GFP+ vs. GFP- comparison.

Strikingly, GFP+ TSCM exhibited a significant downregulation of cytotoxic gene signature. PCR and intracellular staining for Granzyme B and CCL5 confirmed that this differential gene expression was specific to TSCM.

HIV-Vpu is known to inhibit NF-κB, a transcription factor involved in the expression of cytotoxic genes. We hypothesized that Vpu might mediate this suppression in TSCM. However, infection with a Vpu-deficient HIV strain had no impact, indicating that this impairment of cytotoxic function occurs through a Vpu-independent mechanism.

Collectively, these findings suggest a specific disruption of the cytotoxic potential of CD4+ TSCM cells by HIV or a preferential infection of TSCM in a less differentiated state. Further studies will unravel potential implications for HIV persistence and immune recovery in HIV infected individuals.

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A Multi-Omics approach to unveil West Nile Virus interplay with the cellular host

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West Nile Virus (WNV) is a mosquito-borne flavivirus causing an infection exhibiting a wide range of symptoms, from mild febrile illness to severe neuroinvasive diseases. In Italy in 2024, 460 cases for human infection have been reported, out of which 272 showed neurological symptoms. In addition, 26 outbreaks were reported in equids and > 350 cases were reported in birds. Overall, the virus is considered now endemic in several Italian regions. Understanding the molecular mechanisms and host-pathogen interactions underlying WNV infections is crucial for developing effective therapeutics and preventive measures. To this aim we applied a multi-omics approach integrating transcriptomics, proteomics, metabolomics, and lipidomics to gain a comprehensive view of the complex biological processes involved in WNV pathogenesis. Transcriptomic analysis revealed 903 up-regulated, predominantly associated with immune response pathways, and 64 down-regulated genes. The proteomics analysis showed mostly up regulated proteins and overlapping with transcriptomic findings, emphasizing a coordinated antiviral response. Metabolomic profiling, supported by the corresponding gene expression and protein abundance data, indicated a metabolic shift from oxidative phosphorylation to glycolysis, possibly aiding viral replication. The lipidomics analysis demonstrated alteration in several lipid classes (e.g. triglycerides, phosphatidylethanolamines, and sphingomyelins) suggesting an altered lipid homeostasis that could also facilitate viral replication. Overall, the integration of the data from the different omics provided a system-level view of the cell-response to WNV infection, highlighting the possible involvement of novel genes and protein and suggesting new insights into host-pathogen mechanisms.

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Emerging Pathogens: Unveiling the Impact of Climate Change on Global Disease Dynamics

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The global rise of mosquito-borne diseases reflects a profound shift in the ecological balance driven by climate change. Warmer temperatures, altered rainfall patterns, and increased humidity are expanding the geographic range and seasonal activity of key mosquito vectors, particularly *Aedes aegypti*, *Aedes albopictus*, and *Culex* species. These changes are fueling the emergence and re-emergence of arboviruses such as dengue, chikungunya, Zika, West Nile virus, and Oropouche virus in regions previously considered unaffected. As transmission patterns become increasingly unpredictable, integrating pathogen genomics with environmental and ecological data is proving essential to understand and anticipate outbreaks. Genomic surveillance enables the tracking of viral evolution and spread, while climate-informed models can identify areas at risk before local transmission becomes established. Evidence from recent outbreaks in Latin America and Southern Europe highlights how climate variability, coupled with urbanization and mobility, is accelerating the spread of these pathogens. Mosquito-borne diseases are no longer confined to the tropics; they are now part of a broader global health challenge shaped by our changing climate. Recognizing these connections is key to building responsive surveillance systems, guiding targeted vector control, and developing strategies that adapt to rapidly evolving transmission landscapes.

Calcium-conducting viroporins in viral replication and pathogenesis

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Viroporins are small transmembrane viral proteins that form ion channels essential for multiple stages of the viral replication cycle, enhancing viral efficiency and pathogenesis. Among them, pUS21, the first viroporin identified from human cytomegalovirus (HCMV) and an evolutionary homolog of cellular TM6SF2, represents a particularly significant model. Localized in the endoplasmic reticulum (ER), it functions as a Ca^{2+} -permeable channel whose release into the cytoplasm activates store-operated calcium entry (SOCE), protects the cell from pro-apoptotic stimuli, increases ATP levels, and promotes cell adhesion and migration through interaction with talin-1 and activation of the Ca^{2+} -dependent protease calpain-2 [Lukanini et al. PNAS 2018; mBio 2023]. A comprehensive proteomic analysis also identified specific protein-protein interactions between pUS21 and host factors, suggesting a role in modulating metabolic and signaling pathways. In parallel, we investigated the Envelope (E) protein of SARS-CoV-2, also localized primarily in the ER, which conducts both monovalent (Na^+ , K^+ , H^+) and divalent (Ca^{2+}) ions. In human cell lines with stable, inducible expression via a TetOn system, we confirmed its Ca^{2+} -permeable channel activity and observed a concurrent increase in intracellular ATP. The use of these cellular system allowed us to map potentially critical protein-protein interactions in this case as well. Despite differences in viral genomes (DNA vs RNA) and amino acid sequences, pUS21 and E share a convergent evolutionary strategy: alteration of host-cell calcium homeostasis. Our study introduces functional data to dissect viroporin-host interactions, laying the foundation for the development of selective antiviral strategies aimed at inhibiting viroporin channel function.

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Plenary Session 6

Phage Biology: from basics to applications

Of phages and flames: virus-host interactions in inflammatory bowel disease

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Viral communities (viromes) are integral components of microbiomes. I will present our recent work on characterising the human blood virome, which is rich in bacteriophages (phages) including several also present in the intestinal virome. Our findings show distinct alterations in the blood virome of Crohn's disease patients compared to healthy individuals, despite the absence of significant variations in their faecal viromes. In addition, we demonstrate that phages can cross intestinal tissues, including epithelial and endothelial cells, via endocytosis, and that this process is enhanced under conditions of barrier dysfunction. I will discuss how these results represent a new microbial signature of Crohn's disease and contribute to our understanding of gut phage dynamics in inflammatory conditions.

Elucidating key steps and players in the adsorption of DEV phage to its *Pseudomonas aeruginosa* host

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Phage therapy is experiencing a resurgence of interest as a promising therapy for curing multidrug-resistant bacterial infections, although its clinical application remains limited and largely experimental. One significant challenge hindering phage therapy development lies in the modest knowledge of the biology of the bacteriophages employed, which limits the rational selection and engineering of phages for therapeutic applications. We have characterized the adsorption strategy of DEV, a component of a therapeutic phage cocktail able to cure *Pseudomonas aeruginosa* infections in different animal models. DEV is a podovirus belonging to the *Schitoviridae* family, whose first isolated member is the N4 phage of *Escherichia coli*. DEV uses the O-antigen moiety of lipopolysaccharide (LPS) as its primary receptor, recognized by the long tail fiber protein gp53. Notably, DEV can also infect deep-rough *P. aeruginosa* strains that produce truncated LPS lacking the O-antigen, suggesting the use of an alternative receptor. Our data point to the essential outer membrane protein LptD as this secondary receptor, with the receptor-binding protein (RBP) gp54 mediating the interaction. gp54 is encoded within the essential *gp56–gp55–gp54* operon, where *gp56* codes for a tail-sealing short fiber. Intriguingly, DEV and N4 both exploit a surface glycan and the corresponding transporter for host recognition and their RBPs share structural similarity. To our knowledge, this represents the first report of a *P. aeruginosa* phage utilizing the essential outer membrane protein LptD as a receptor.

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Lytic bacteriophages against *Klebsiella pneumoniae* and their impact on bacterial virulence

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The use of lytic bacteriophages as therapeutic agents for the treatment of human infections is regaining interest, especially due to the spread of antibiotic-resistant bacteria. Beyond directly killing prokaryotic cells, phages also exert a selective pressure that can promote the selection of lysis-resistant cells during treatments. This phenomenon typically arises from modifications of phage receptors, often located on bacterial surface, which are involved in early virus-bacterium interactions. Here, we aimed to i) obtain *in vitro* phage-resistant derivatives, ii) characterize the genetic mechanisms underlying this phenotype and iii) evaluate changes in interactions with the host immune system.

The vB_KpM_GP-7 phage was isolated from wastewater using the BO-FR-1 *Klebsiella pneumoniae* strain as host. This strain is a member of the *K. pneumoniae* ST258 clade II clonal lineage, previously obtained by infection at high multiplicity with the ϕ BO1E phage of the KKBO-1 clinical strain. Three independent vB_KpM_GP-7 resistant derivatives have been characterized by genome sequencing and by evaluating their virulence on human macrophages and susceptibility to human serum.

The vB_KpM_GP-7 resistant strains were all characterized by different alterations of genes involved in the lipopolysaccharide biosynthesis pathway, suggesting that components of this

macromolecule are the receptors used by this phage to start an infective cycle. Phage resistant derivatives were all more susceptible than the parental clinical strain to the action of complement-active human serum and to macrophage killing, even if differences were observed among the tested strains.

These results underscore that selective pressure exerted by phages could lead to decreased virulence in *K. pneumoniae*.

Liaisons dangereuses: lysogenic phages, antibiotic resistance, virulence and horizontal gene transfer in *Streptococcus pyogenes*

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The polylysogenic nature of *Streptococcus pyogenes* genomes, which may harbor up to eight prophages, complicates the study of individual phage properties. Here, we use Φ 1207.3—an atypical *Caudoviricetes* of *S. pyogenes*—as a paradigm to dissect the interplay between lysogeny, antibiotic resistance, and genetic exchange. Unlike canonical phages, Φ 1207.3 does not form plaques, exhibits low burst activity, and carries the *mef(A)-msr(D)* macrolide resistance gene pair. Transfer of Φ 1207.3 into a prophage-free *Streptococcus pneumoniae* host revealed its capacity to produce mature virions upon mitomycin C induction, albeit at low titers ($\sim 10^4$ particles/mL of culture supernatant). Functional assays demonstrated efficient lysogenization (7.5×10^{-6} lysogens/recipient) but minimal lytic activity, suggesting evolutionary specialization for temperate survival. Immunoassays targeting Φ 1207.3 major capsid protein revealed phage particle association with bacterial surface, implicating that Φ 1207.3 transfer might need cell-to-cell contact. Further, Φ 1207.3 encodes an SOS-like cassette that enhances both bacterial UV survival (34-fold) and mutagenesis (18-fold). Φ 1207.3 is an atypical mobile genetic element that shapes *S. pyogenes* evolution through dual roles: disseminating antibiotic resistance and modulating host mutagenesis.

Phages in the dark: exploring the viral diversity and functional potential in subterranean environments

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Viruses, including bacteriophages, are abundant and highly diverse across Earth's ecosystems. They play fundamental roles in shaping microbial community structure and function through mechanisms such as lytic activity, horizontal gene transfer, and host metabolic reprogramming. However, phage diversity, abundance, and ecological roles remain largely unexplored in extreme and understudied environments. Caves are known to host an extraordinary microbial diversity despite the absence of light and extreme oligotrophic conditions. In caves, microorganisms often depend on chemolithotrophy and mutualistic networks to sustain the development of complex communities, being at the foundation of food webs.

In this study, we conducted a large-scale metagenomic analysis of phages in cave environments to investigate the link between phage diversity, microbial evolution and functional potential. We analysed over 100 metagenomic datasets from a wide range of cave types and sample matrices

(e.g., biofilms, soil, and water). Using a suite of bioinformatic tools, we identified and classified viral sequences, conducted comparative analyses, and performed functional annotations. Our results reveal that viral community composition and functional profiles vary significantly across cave types and differs from other environments. Analysis of metagenome-assembled genomes (MAGs) from unculturable cave bacteria further revealed prophages encoding genetic traits potentially linked to environmental adaptation, colonization, and persistence. This work advances our understanding of the Earth's virosphere and sheds light on the ecological and evolutionary roles of phages in subterranean microbial ecosystems.

PARALLEL SESSIONS: short talks from selected abstracts

Session A - Microbial genetics and genomics

Chairs: R. Isticato, A. Martorana

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A1	The <i>Pseudomonas aeruginosa</i> sirB2 gene controls fitness in anaerobic conditions and its inactivation leads to increased virulence and small colony variants emergence via secondary mutations in the wsp operon.
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Pseudomonas aeruginosa is the leading cause of death in cystic fibrosis (CF) patients, yet the genetic mechanisms driving its fitness in the host remain poorly defined. Previously collected transcriptomic data of clinical samples showed that the expression of the gene PA14_RS04555 (sirB2) is stimulated in the CF lung environment. In this work, we show that sirB2 is regulated by the global transcriptional regulators Vfr and AmrZ.

Loss of sirB2 markedly enhanced *P. aeruginosa* pathogenicity, increasing virulence in *Galleria mellonella*, and promoting bacterial translocation and biofilm formation in a differentiated airway epithelial infection model. Deletion of *sirB2* triggered the emergence of biofilm-proficient rugose small colony variants (RSCVs), driven by elevated c-di-GMP and increased Pel polysaccharide production, particularly when cultures were grown in an oxygen-limited environment. The RSCV

phenotype depends on suppressor mutations in the *wsp* operon, in response to redox imbalance caused by the lack of *sirB2* under oxygen-limited conditions. Consistent with this observation, the *sirB2* mutant exhibited impaired fitness during anaerobic respiration when nitrate was the sole electron acceptor, in a manner independent of the ubiquinone pool.

Our findings show that *sirB2* inactivation promotes RSCV emergence and identify *sirB2* as a novel genetic determinant of redox homeostasis and metabolic fitness in host-relevant conditions, thus underlining the role of redox balance in chronic CF infections.

A2

Hydroxyl radical generation by PirA connects β -oxidation to oxidative stress: an evolutionarily conserved mechanism?

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Pirins are evolutionarily conserved, iron-containing proteins implicated in various stress-associated cellular functions. In prokaryotes, Pirins are widely distributed across taxa and often occur in multiple copies, although few have been functionally characterized. In *Streptomyces ambofaciens*, the Pirin-like protein PirA acts as a redox-sensitive negative modulator of AcdB, a very long-chain acyl-CoA dehydrogenase (vLCAD) involved in the first step of β -oxidation. A taxonomic classification of Pirins across prokaryotic and non-prokaryotic organisms revealed a strong association between Pirin occurrence and aerobic energy metabolism. Additionally, an AI-based reclassification of Pirin-like proteins in *Streptomyces* identified seven distinct groups, four of which are predominant. Comparative genomic analysis of 929 *Streptomyces* strains identified 330 conserved genes of unknown function, many linked to transcription and lipid metabolism, highlighting the importance of *pirA*, *acdB*, and proteins related to lipid metabolism in *Streptomyces*. To investigate a potential role in antioxidant regulation, a *pirA*-deficient mutant of *S. ambofaciens* was analyzed. This mutant exhibited elevated intracellular H₂O₂ levels during vegetative growth, partially attributed to increased AcdB activity, which produces H₂O₂ as a byproduct. Despite the oxidative stress, expression of antioxidant genes—including *catA* (encoding the vegetative catalase) and *catR* (its regulatory gene)—was reduced. In Gram-positive bacteria, CatR represses *catA* transcription and is inactivated by hydroxyl radicals, leading to derepression.

In vitro assays showed that PirA reacts with H₂O₂ to produce hydroxyl radicals and interacts with CatR, suggesting that PirA facilitates CatR inactivation and promotes *catA* expression in response to oxidative stress.

A3

The *ydaJKLMN* operon mediates the adhesion properties of *Bacillus subtilis* spores

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Probiotic spores of *Bacillus subtilis* SF106 (licensed by SAVIO s.r.l.) bind human mucin more efficiently than spores of other strains of the same species¹. The adhesion of SF106 spores to biotic and abiotic surfaces was compared with that of spores of two *B. subtilis* type strains, 168 and NCIB 3610, and of the lab collection strain PY79. Such analysis indicated that spores of SF106,

168 and NCIB 3610 bound both biotic or abiotic surfaces with a similar efficiency, significantly higher than that observed with PY79 spores. A comparative genomic analysis identified a 17 kb region as the only chromosomal part lacking in PY79 and present on the chromosomes of all other three strains, suggesting that one or more genes contained in the 17 kb were involved in modulating the efficiency of spore adhesion. Bioinformatic analysis of the 17 kb region indicated the presence of the about 7 kb *ydaJKLMN* operon, coding for a putative glycosyl hydrolase (*ydaJ*), a c-di-GMP receptor (*ydaK*) and for three enzymes involved in the production/secretion of an exopolysaccharide required for cell aggregation and biofilm formation (*yLMN*)². Spores of a SF106 mutant strain lacking the YdaJ-N proteins showed a reduced adhesion efficiency, similar to those of PY79 spores. Conversely, the introduction of *ydaJ-N* operon of 168 in PY79 increased the adhesion efficiency of spores to levels of SF106, 168 or NCIB 3610 spores. A detailed analysis of *ydaJ-N* expression is in progress.

¹Int. J. Mol. Sci. 2022; **23**:14946

²Environ. Microbiol. Rep. 2017; **9**:211-222

A4 Starving the invader: disrupting iron uptake to defeat *Staphylococcus aureus*

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The rapid emergence of antibiotic-resistant *Staphylococcus aureus* strains underscores its role as a key contributor to the global antimicrobial resistance (AMR) crisis. A key factor in its virulence and persistence during infection is the ability to acquire iron, an essential nutrient restricted by the host's defence mechanism known as nutritional immunity. To circumvent host-imposed iron limitation, *S. aureus* employs two sophisticated iron acquisition systems: the iron-regulated surface determinant (Isd) system, which scavenges heme-bound iron directly from host hemoglobin (Hb), and the siderophore-mediated system, which captures iron from host iron-binding proteins via small chelators, primarily staphyloferrin B (SB), only produced by the most invasive, coagulase-positive *S. aureus* strains.

Here, we present two innovative strategies targeting *S. aureus* iron metabolism with potential therapeutic applications. The first involves a newly-identified compound (C35)¹ that selectively binds Hb and inhibits its interaction with the IsdB receptor. Using both wild-type *S. aureus* and its isogenic in frame-deletion mutant (Δ *isdB*), C35 was shown to markedly inhibit *S. aureus* growth by blocking hemophore-mediated iron uptake, an essential pathway for survival during infection. The second approach targets SbnA, a key enzyme in SB biosynthesis. Searching for citrate (*i.e.*, SbnA physiological inhibitor) analogues, the methyl esters of 2-phenylmaleic acid (2-PhMA) and of 2-phenylsuccinic acid (2-PhSA) were found to impair siderophore-dependent iron acquisition in *S. aureus*, and its subsequent growth under iron starvation².

Together, these dual approaches targeting both heme- and siderophore-mediated iron uptake pathways provide a robust foundation for the development of next-generation antimicrobial agents against *S. aureus* multidrug-resistant strains.

¹Cozzi M, Failla M et al. Identification of small molecules affecting the interaction between human hemoglobin and *Staphylococcus aureus* IsdB hemophore. *Sci Rep.* 2024 Apr 9;14(1):8272. doi: 10.1038/s41598-024-55931-8.

²Hijazi S, Cozzi M et al. First-in-class inhibitors of SbnA reduce siderophore production in *Staphylococcus aureus*. *FEBS J.* 2025 Apr 2. doi: 10.1111/febs.70076.

A5

The Role of Teichoic Acids in Bifidobacteria in Mediating The Interaction with the Human Host

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Teichoic acids (TAs) are cell wall-associated polymers known to influence both microbial physiology and host interaction. However, their role within the genus *Bifidobacterium* remains largely uncharacterized. An *in silico* survey of more than 3000 publicly available bifidobacterial genomes, revealed significant inter-species variability in the genetic determinants involved in TA biosynthesis. Interestingly, only a limited number of bifidobacterial species harbored a conserved gene cluster predicted to be essential for the synthesis and assembly of TAs, with *Bifidobacterium bifidum* emerging as a prominent example. Focusing on three strains of *B. bifidum*, including the PRL2010 reference strain of this species, we compared transcriptomic profiles following exposure to human intestinal epithelial cells and observed strain-dependent differences in the expression of TA-related genes, suggesting a host-influenced and strain-specific transcriptional response. Functional validation through *in vitro* experiments using isogenic *B. bifidum* PRL2010 mutants, defective in a key TA biosynthetic locus, confirmed a role for TAs in modulating adhesion to epithelial cells and influencing interactions with human macrophages. Moreover, *in vivo* data from murine colonization models demonstrated upregulation of these TA-related genes, further supporting the notion that host signals modulate the expression of TA biosynthetic pathways in *B. bifidum*. In conclusion, these findings point to a potential role for TAs in mediating microbe-host cross-talk and underline the species-specific strategies employed by bifidobacteria to interact with the intestinal environment.

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A6

memod-s: a standardised workflow to explore and analyse prokaryotic methylation patterns for Nanopore sequencing data

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Understanding the bacterial epigenome is increasingly recognised as essential for uncovering key mechanisms of gene regulation, host-pathogen interactions, and adaptation to environmental changes. Third-generation sequencing technologies, such as Oxford Nanopore, now enable the direct detection of DNA modifications, making genome-wide epigenomic investigations both feasible and cost-effective. However, analysing nanopore sequencing data remains computationally intensive and requires multiple steps, which can be complex to integrate. Currently, no existing workflow combines these steps in a single, easy-to-use pipeline. Additionally, many available tools lack automated genome-wide methylation profiling with integrated visualisations and statistics. We present *memod-s*, a Snakemake-based workflow that integrates multiple state-of-the-art tools to address these challenges. *memod-s* is a modular and user-friendly workflow that simplifies the entire nanopore data analysis process, from basecalling and quality control to genome assembly, annotation, and methylation analysis. By integrating all essential steps into one cohesive pipeline and producing comprehensive genome-wide methylation profiles enriched with graphical visualisations and statistics, *memod-s* reduces the complexity of nanopore data analysis and provides insights into bacterial methylation patterns and their potential biological implications. To better illustrate this software utility, we applied *memod-s* to a real case study, i.e. the analysis *Vibrio aestuarianus* genome and its nucleotide modification patterns, representing, to the best of our knowledge, the first epigenomic investigation of a representative of the genus *Vibrio*.

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A7

Exploring Bacterial Evolution across the Genome Architecture Landscape

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Bacteria are among the earliest life forms and colonise every habitat, including soil, water, and air, even under extreme environmental conditions. This niche exploration left deep genetic signs on bacterial genomes. During the last decades, the remarkable effort in studying bacterial evolution has led to the identification of specific genetic traits associated with phenotypic and/or ecological characteristics. In this work, we shifted our focus to broader genome structural features, such as base composition and gene disposition. We defined a wide set of indices describing several aspects of genome organisation that we used to characterise the genomes of more than 30,000 representative bacterial species. This allowed us to picture out the global landscape of bacterial genome architecture and to discover that all bacterial genomes can be grouped into four main configurations. Then, we investigated how bacteria explored this genome

architecture landscape during their evolution, exploiting the phylogenetic relationship among the species. The evolutionary reconstruction of the genomic configurations revealed major transitions in the genome organisation of different bacterial groups such as Saccharimonadia, Bacteroidales, Bacilli, Negativicutes and Clostridia. Overall, these results indicate a preference for major genome configurations in bacteria and highlight the preferential evolutionary paths followed by bacteria throughout their history, suggesting that genome architecture could change in association to external factors, such as ecology and adaptation.

A8

Conjugative CRISPR-Cas vector to cure KPC-encoding plasmids in *Klebsiella pneumoniae*

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Introduction

Conjugative vectors are efficient delivery systems for introducing genes into bacterial strains. We used a 65 kb self-conjugative, low-copy-number plasmid (R69#1), to deliver the CRISPR-Cas9 system.

Material and Methods

E. coli strains carrying the CRISPR-Cas9 vector without sgRNA guides (A3) or with sgRNAs targeting the *bla*KPC gene (G6) were conjugated with *E. coli* strains with *bla*KPC-pKpQIL plasmid, or with clinical KPC-producing *Klebsiella pneumoniae* (Kp) strains of the ST37, ST512, ST111, ST101, ST2502 sequence types, harboring different plasmid content.

Results

Conjugation efficiency of R69#1 derivatives was very high in both *E. coli* and Kp (1×10^{-1} – 1×10^{-2}), except for ST111-Kp (1×10^{-6}). In *E. coli*, KPC-plasmids were efficiently cut by CRISPR-Cas9 with guides (G6), measuring a 3-Log reduction of KPC-producing strains in the conjugant population. End-Joining Repair (EJR) was observed in *E. coli* conjugants. In Kp, a 2-Log reduction of KPC-positive conjugants was observed in G6/KPC vs A3/KPC conjugants. Additional loss of $2.5\text{--}5 \times 10^3$ KPC-conjugants was observed after 24-hour of growth of the conjugation mixture without antibiotics. This loss was attributed to post-segregational killing (PSK) occurring in the hours following the CRISPR-Cas9 cut in KPC-plasmids stabilized by Toxin-Antitoxin systems. No EJR was observed in Kp conjugants.

Conclusions

CrispCas9-armed conjugative vectors help in studying complex plasmid contents in microbial populations. In this application the vector has been successfully used to target Kp plasmids carrying the *bla*KPC gene, gaining an interesting model of PSK in a bacterial species with no EJR system.

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A9	Molecular mechanisms implicated in the resuscitation of <i>Acinetobacter baumannii</i> Viable But Non-Culturable (VBNC) cells induced by desiccation
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Recent studies have demonstrated that the opportunistic bacterial pathogen *Acinetobacter baumannii* withstands the desiccation stress by entering the Viable but Non-Culturable (VBNC) state. The VBNC state is a reversible condition in which, following exposure to stress, a subpopulation of viable microbial cells loses culturability in nutrient media. The process by which the VBNC cells regain culturability is called resuscitation. While some mechanisms involved in desiccation resistance have been interrogated, the molecular pathways enabling the resuscitation of *A. baumannii* VBNC cells remain largely unknown. This study aims to elucidate the molecular mechanisms responsible for the resuscitation of *A. baumannii* VBNC cells following desiccation. High-throughput sequencing-mediated transcriptome profiling (RNA-seq) was performed to characterize the transcriptomic profile of *A. baumannii* across three stages: before desiccation, after 1-week desiccation, and post-resuscitation. Transcriptomic analysis revealed that 75 genes were downregulated during desiccation and subsequently upregulated during resuscitation. Among them, *uvrA* was one of the most significantly induced genes in the resuscitation, but not after desiccation. *In silico* sequence and structural analyses revealed that *A. baumannii uvrA* shares high similarity with *uvrA* from other bacterial pathogens. UvrA is involved in the UvrABC DNA damage repair system, and its function in *A. baumannii* was validated by exposing a *uvrA* knockout strain to UV-C light and oxidative stress-induced DNA damage. Finally, desiccation and resuscitation assays with an *A. baumannii uvrA* knockout mutant strain demonstrated that UvrA plays an essential role in the recovery of *A. baumannii* from the VBNC state induced by desiccation.

A10	Role of DedA proteins in <i>Pseudomonas aeruginosa</i>
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The peptidoglycan biosynthetic pathway represents a major antibacterial target, with a key step consisting in the recycling of undecaprenyl phosphate (C55-P) across the cytoplasmic membrane. This lipid carrier is involved in transporting various cell envelope components, including peptidoglycan precursors and sugars such as 4-amino-4-deoxy-L-arabinose (L-Ara4N), whose attachment to LPS represents the main polymyxin resistance mechanism in several bacteria. Recent studies proposed DedA family proteins as responsible for flipping C55-P back during the recycling process in some Gram-positive and Gram-negative bacteria, but they have never been studied in *Pseudomonas aeruginosa*. Here, we investigated the role of DedA proteins in this pathogen. Six *dedA* genes were identified, but only the deletion of two, PA4011 and PA4029, caused phenotypic defects in the reference strain PAO1. Specifically, the absence of PA4029 increased fosmidomycin sensitivity, resensitized a colistin-resistant recombinant strain that constitutively produces L-Ara4N-modified LPS, and significantly reduced the ability to

develop colistin resistance; all these phenotypes are consistent with impaired C55-P recycling. The Δ PA4011 mutant was instead more susceptible to the cation-chelating agent EDTA, and its sensitivity to EDTA (and fosmidomycin) was increased when the gene encoding the C55-PP phosphatase UppP was concomitantly deleted. Overall, our results suggest that PA4011 and PA4029 may act in C55-P(P) recycling: PA4011 as a C55-PP phosphatase, since it also contains a PAP2-like domain, and PA4029 as a C55-P flippase. Genomic and cross-complementation analyses suggest that, while PA4011 is highly conserved in the *Pseudomonas* genus, PA4029 has been functionally replaced by a DUF368 protein in some *Pseudomonas* species.

A11

Repurposing FDA approved drugs to disrupt *Mycobacterium abscessus* iron acquisition pathway by targeting the salicylate synthase Mab-SaS.

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The nontuberculous *Mycobacterium abscessus* (*Mab*) is a rapid-growing opportunistic pathogen that causes severe lung infections in individual with chronic conditions. Its high intrinsic tolerance to antibiotics along with its capability to rapidly acquire new antimicrobial resistance mechanisms increase the difficult of treatments with existing therapies, underscoring the urgent need for new effective strategies. One alternative strategy to fight *Mab* is focusing on its virulence factors, such as the iron acquisition mechanism mediated by mycobacterial siderophores. *Mab* siderophores biosynthesis begins with the conversion of chorismic acid into salicylate catalyzed by the attractive pharmaceutical target enzyme salicylate synthase (SaS).

The development of novel drugs can be a long process, therefore we adopted a repurposing strategy to accelerate the discovery of effective treatments, aiming to identify existing approved drugs capable of inhibiting this target. The *in silico* screening of three databases of FDA-approved drug against the SaS crystal structure identified eleven potential ligands, that were selected for *in vitro* assays on the recombinant enzyme. Fostamatinib, hydroxystilbamidine, and esomeprazole emerged as potent competitive inhibitors, exhibiting IC₅₀ values in the order of low micromolar concentration. Notably, hydroxystilbamidine also demonstrated moderate activity in a *Mycobacterium smegmatis* model in iron limiting conditions, suggesting its potential for combination treatments with antibiotic to enhance therapeutic efficacy.

These findings highlight the potential of drug repurposing as a viable approach to fight *Mab* infections. Further optimization of these inhibitors could contribute to the development of more effective therapies against NTM infections.

A12

Genome analysis and mobilome characterization of *Trichomonas vaginalis*-associated *Mycoplasma hominis* isolates through whole genome sequencing

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Mycoplasma hominis is a human opportunistic pathogen which can establish *in vivo* an intracellular symbiotic relationship with the sexually transmitted parasite *Trichomonas vaginalis*. Here, we investigated the genomic features and mobilome profile of seven *T. vaginalis*-associated *M. hominis* isolates. Complete genome sequencing was obtained with Nanopore technology, mobilome functional analysis with qPCR. Genomes ranged in size from 696- to 748-kbp, mobilome accounts up to 10% of genome and includes (i) ICEHo-I, (ii) ICEHo-II, (iii) prophage MHoV-1, and (iv) insertion sequence ISMhom-1. All elements produce excised circular forms with frequencies ranging from 1.11×10^{-5} to 1.41×10^{-2} copies/chromosomes, and *attB* site restoration from 1.2×10^{-7} to 4.03×10^4 copies/chromosomes. Elements do not carry antibiotic resistance genes, while a total of 36 SNPs were found in the *gyrA*, *gyrB*, *parC*, *parE* genes including two known fluoroquinolones resistance mutations resulting in V417I and K144R substitutions in ParE and ParC, respectively. All *M. hominis* isolates contained the chromosomal virulence genes *vaa*, *oppA*, *tuf*, *hlyA*, *hlyC* and MHO_0730 nuclease, moreover 3 isolates harboured both *alr* and *goiC* genes, while one isolate only *alr*. Significant genomic variability was found in the phase variable adherence-associated adhesin *vaa* gene and in its downstream high-recombining genomic region. ICEHo-I integrates downstream *rplA* gene, whereas ICEHo-II in 3 different loci within the same genome including the *vaa* downstream region. Prophage MHoV-1 integrates in various sites located downstream lipoprotein encoding genes or at the 3' end of *ychf* gene. *M. hominis* genome analysis highlighted the plasticity and evolution of mycoplasma genomes with increasing DNA acquisition via horizontal gene transfer.

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Session B - Environmental and industrial microbiology

Chairs: **E. Bona, T. Rinaldi**

B1	Dissecting molecular, physical, and chemical factors of wasps' gut shaping <i>Saccharomyces cerevisiae</i> survival
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The yeast *Saccharomyces cerevisiae*, a long-standing model organism, has revealed a surprising evolutionary partnership with social wasps (*Polistes*, *Vespa*, and *Vespula*). Beyond acting as passive vectors or overwintering reservoirs, wasps provide a dynamic microhabitat in which inter-yeast strain reproduction (i.e., outbreeding), an infrequent but evolutionary crucial process, occurs.

Our multidisciplinary study has explored the wasp alimentary canal to assess the features that make this environment promote yeast outbreeding. Using fluorescent nanosensors, we characterized the gut microenvironment to gather information on physico-chemical conditions (pH, sugar, viscosity), and used the obtained data to simulate the wasp crop and gut features in laboratory assays to examine their impact on yeasts. In parallel, we performed genomic analyses of both host and yeast to unravel the molecular framework of this interkingdom interaction. Furthermore, we have shown that the gut, particularly different from the stomach, promotes ascus rupture and spore germination, key steps that allow mating between different yeast strains.

This integrated approach, bridging ecology, microbiology, and genetics, has shed light on a poorly understood but powerful driver of yeast evolution. This research highlights the importance of insect hosts as active agents in microbial diversification and opens new avenues to understand how environmental factors shape the life cycle and adaptation of microbial species in nature.

B2	Climate change impact on soil microbial communities of the West Antarctic Peninsula
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The West Antarctic Peninsula (WAP) is one of the fastest warming regions of the planet, and may be fast approaching a climatic tipping point^[1,2]. Measurements and climate models indicate vulnerabilities to the prevalence and stability of frozen ground and permafrost. Microorganisms

are key players in the mobilization of carbon and nutrients in global soils, especially in Antarctica where there is an almost complete absence of higher-order plants. While the role of microorganisms in contributing to and regulating greenhouse gas fluxes in the Arctic has been extensively investigated, less is known about their role and activity in thawing Antarctic permafrost. Here we present data from 8 terrestrial sites along the WAP, where the past 7 years have been the seven warmest on record^[2]. We measured soil surface temperatures of between 2.3 and 17.1 °C, with an average of 8.5 °C. We characterized soil microbial taxonomic and functional diversity using shotgun metagenomics correlated with geological settings and soil geochemistry, as well as the interstitial soil gas composition. We found pronounced differences in soil microbial communities between the different sample areas, and a correlation soil temperatures. We reveal that the microbial communities inhabiting soils on the Antarctic peninsula are capable of degrading complex refractory organic matter, potentially contributing to greenhouse gas emission. Our study provides important insights into anticipated climate change-induced developments of Antarctic soil microbial ecosystems, revealing their possible future contribution to greenhouse gas emission and biogeochemical cycling in a warming Antarctica

^[1]Masson-Delmotte, V. et al. (eds) IPCC (Cambridge University Press, 2021)

^[2]Gorodetskaya, I.V., Durán-Alarcón, C., González-Herrero, S. et al. *npj Climate and Atmospheric Science* 6, 202 (2023)

B3

Extracellular Membrane Vesicles from *Streptomyces violaceoruber*: a promising natural strategy for enhancing plant growth and health

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Climate change and the growing demand for food necessitate eco-sustainable solutions to optimize resource use and reduce chemical inputs. In this context, biofertilizers, based on living Plant Growth Promoting (PGP) bacteria, offer a promising approach. Among PGP bacteria, *Streptomyces* species show significant potential. Streptomycetes are well known for their morphophysiological differentiation and production of bioactive compounds. Extracellular membrane vesicles (eMVs) - nanostructures naturally released by cells – can play key roles in intercellular communication, carrying proteins, nucleic acids, and other bioactive molecules. Therefore, this study focuses on the characterization of eMVs isolated from the PGP bacterium *Streptomyces violaceoruber*, evaluating their morphology, cargo, and biological effects.

eMVs were isolated through ultracentrifugation from *S. violaceoruber* six-day-old cultures grown in liquid minimal medium. Morphological, dimensional and quantitative characterizations were performed through scanning transmission electron microscopy, dynamic light scattering and nanoparticles tracking analysis. Molecular profiling was performed using omics-based approaches (*i.e.* next-generation sequencing, mass spectrometry analyses).

eMV, ranging from 100–200 nm in size, contain RNA and the whole *S. violaceoruber* genomic DNA. Additionally, they contain proteins, but their metabolic cargo has yet to be fully characterized. Interestingly, eMV exhibited antibacterial properties and showed PGP effects on tomato seedlings. Experiments are underway to evaluate their capability to mitigate plant biotic stresses, testing their effect against phytopathogens (*i.e.* *Verticillium* sp.) infections.

Therefore, this research aims to provide new evidence on the role of *Streptomyces*-derived eMV in trans-kingdom communication (microbe-microbe and plant-microbe interactions), and to lay the groundwork for the development of new eMV-based biofertilizers.

B4 Plant growth-promoting Actinomycetota modulate bioactive metabolites profiles in mediterranean plantsg

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Plant-derived bioactive compounds are abundant in Mediterranean plant species like oregano and tomato, offering valuable applications in human health-related fields. Microbial-based biostimulants (MBBs), particularly those rich in microbes from the phylum Actinomycetota (previously Actinobacteria), represent a promising strategy to modulate both the quantity and quality of bioactive compounds in the inoculated plants. In this study, two soil Actinomycetota - *Streptomyces violaceoruber* and *Kocuria rhizophila* - were assayed as Plant Growth-Promoting Bacteria (PGPB) on oregano and tomato plants under controlled laboratory conditions and greenhouse cultivations, while open field experiments were carried out only for oregano. The aim was to assess their influence on plant growth and metabolite yield. Overall, the results show that these two bacteria positively influenced several morpho-physiological parameters of the investigated plants: inoculations improved germination rate, shoot heights, and chlorophyll content in oregano and shoot heights, leaf area and biomass yield in tomato. Furthermore, both species exhibited significant changes in their metabolic profiles following PGPB inoculations. Indeed, in oregano, Gas Chromatography-Mass Spectrometry (GC-MS) analyses revealed a modulation in terpenes production, both in flower volatilome and essential oils, while, in tomato, an increased proline content was detected. Transcriptomic analyses suggested a different involvement of the two Actinomycetota in the modulation of tomato biosynthetic pathways. These results provide evidence that *S. violaceoruber* and *K. rhizophila* enhance plant growth and modulate the metabolome in oregano and tomato plants. Therefore, they represent promising tools for improving crop performance and, potentially, for the targeted modulation of secondary metabolite production in mediterranean plants.

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B5	Study of microbial diversity associated to <i>Helichrysum microphyllum</i> subsp. <i>tyrrhenicum</i>, a metallophyte endemic of Sardinian mining areas
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Mine tailings represent a large-scale issue requiring the development of a sustainable remediation management plan with a multifactorial approach. In the Iglesiente region, past mining activities left severe contamination of Zn, Pb and Cd. Among bioremediation technologies, phytoremediation appears to be a promising strategy owing to the use of autochthonous plant species and their associated microorganisms naturally adapted to these harsh conditions. Therefore, the study of microbiome in rhizosphere and roots of native plants is crucial for the development of a successful remediation strategy. In this study, we investigated the rhizosphere and root microbiomes of *Helichrysum microphyllum* subsp. *tyrrhenicum*, a metallophyte endemic of Sardinian mining areas. Sampling was conducted in three zones based on proximity to a mine tailing deposit: outside, at the border, and inside the dump. Rhizosphere and bulk substrates were collected, and different parameters were analysed: physico-chemical properties, microbial activity via dehydrogenase assay, microbial functional diversity using BILOG system, bacterial and fungal communities by high-throughput sequencing. For both substrates, metals levels at the border and inside the dump were significantly higher than outside. Dehydrogenase assay showed a statistically significant increase in microbial activity from inside to outside the dump. Currently, analysis of microbial community by high-throughput sequencing and multivariate analysis are in progress. Our results highlight the importance of plant-associated microbiomes in metal-contaminated environments and support their relevance for site-specific remediation strategies. This work has been developed within the framework of the project e.INS www.einsardinia.eu (Next Generation EU- PNRR-M4 C2 I1.5 CUP F53C22000430001).

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B6	Preliminary characterization of the microbial community living in a multi-pond solar saltern located in south Sardinia
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Multi-pond salterns are excellent models for studying microbial diversity along salinity gradients. However, microbial communities of hypersaline ecosystems remain largely underexplored despite their biotechnological potential.

We present the first metagenomic study of the active Conti Vecchi saltern (South Sardinia, Mediterranean Sea). Water (1L) and sediment (50g) samples were obtained from three stations in each of four ponds with increasing salinity (4%-30%). DNA was extracted from triplicates and sequenced.

To date, prokaryote taxonomic profiling has been completed for the low- (st.1,2,3) and medium-low (st.4) salinity ponds. Overall, 20,587 species were identified: 409 *Archaea*, 16,375 *Bacteria*, 3,685 *Eukaryota*, and 119 viruses. Among *Bacteria*, *Pseudomonadota* and *Actinomycetota* phyla dominate, consistent with low-salinity communities.

Microbial composition shifts along the salinity gradient, with *Archaea* increasing in relative abundance. Interestingly, the *Halobacteria* class is already dominant in the low salinity pond and increases further in the medium-low one, despite literature reports their prevalence as typically associated with higher salinities, potentially indicating unique local adaptation.

The ponds were also analysed for viral and microbial abundance, nutrient and metal concentrations, and metabolic activity. The results allow us to support the “Piggyback-the-winner” hypothesis, where high host density favours lysogeny over lysis.

We are currently extending the analysis to viral and eukaryotic communities, to compare the obtained data with metagenomes from a disused Sardinian saltern and assess how long-term anthropogenic activity shapes microbial communities in extreme environments.

The project is financed by the E.U. “Next Generation EU” within the PRIN 2022 framework (MARICOSTEMS Project; n°: 2022FLKW8E).

B7 Developing a Drone-Based System for Rapid Bioaerosol Sampling

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Intensive farming practices are a major source of bioaerosols—airborne particles containing bacteria, viruses, and other microorganisms—which can increase the risk of zoonotic and respiratory diseases. Monitoring these airborne microbial communities is challenging due to their spatial and temporal variability and the difficulty in collecting representative samples, often requiring large air volumes over extended periods. This study presents the development of an innovative air monitoring system designed to overcome these challenges. The system utilizes microbial traps mounted on a customized 3D-printed support, integrated into a drone platform for air sampling. A proof-of-concept test was conducted on a cattle farm, where the drone successfully collected microbial material in just 10 minutes of flight. To maximize DNA recovery, a tailored extraction protocol was implemented, incorporating a viral DNA recovery kit. Although DNA yields were low, the use of an optimized nested PCR approach enabled the amplification of high-quality microbial DNA. Subsequent Next-Generation Sequencing (NGS) identified diverse microbial genera typical of agricultural environments, along with potentially pathogenic taxa. These findings demonstrate the system’s effectiveness in capturing airborne microbes and its potential for broader applications in environmental surveillance. The platform is low-cost, customizable, and adaptable, making it suitable for various research contexts. With further optimization, this approach could significantly enhance the study of bioaerosol dynamics and improve early detection of airborne pathogens in high-risk settings like intensive farms.

B8

Spatiotemporal variations of the bacterial communities colonizing five pyroclastic caves located in the Rome metropolitan areaE.P. Riggio¹, A. Basile¹, P. Turrini¹, C. Romano¹, M. Lucidi^{1,2}, P. Visca^{1,2}¹Department of Science, Roma Tre University, Italy²NBFC, National Biodiversity Future Center, Palermo, Italy

Urban caves are subterranean environments resulting from rock extraction in urbanized areas. Owing to their shallow depth, such caves are vulnerable to anthropogenic pressures and environmental contamination. Here, the bacterial colonization of five pyroclastic urban caves located in Rome was analysed over two years (2022–2023). Caves were excavated within volcanic deposits from the Colli Albani complex: Celio, Forlanini, and Monteverde were carved in the Tufo Lionato tuff (TL) unit; Fiume Ipogeo and Romana in the Red Pozzolan (RP) unit. All these caves feature hydrological elements (lakes, wells, or streams).

Three environmental matrices were sampled from each cave: wall rock, sediments, and groundwater, and analysed through 16S rRNA gene sequencing (V5–V6 regions) to characterize bacterial communities across distinct subterranean microhabitats.

The results revealed a diverse and dynamic microbiota, with a core of forty amplicon sequence variants (ASVs) shared across caves. Despite compositional consistency at the phylum level dominated by *Pseudomonadota*, *Bacteroidota*, and *Actinomycetota*, temporal fluctuations emerged, particularly in RP caves, where ASVs of *Limnohabitans* and *Acinetobacter* exhibited significant shifts in abundance over time, likely due to local environmental drivers.

Co-occurrence analyses of key genera within the dataset pointed to the absence of competition between taxa. Aerobic chemoheterotrophy was identified as the most abundant metabolic process. Chemical and lithological analyses revealed that, among heavy metals, arsenic and lead frequently exceeded safety thresholds, likely impacting the microbial community structures. These findings underscore that urban caves host complex microbial communities that can serve as bioindicators for environmental monitoring of urban settings.

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B9

Bioplastic in sandy beaches: the involvement of gut bacterial communities from supralittoral amphipods in polymers degradationA. Russo^{1*}, A. D'Alessandro¹, M. Di Paola¹, B. Cerasuolo¹, S. Renzi¹, N. Meriggi², L. Conti³, S. Santini³, J. Costa⁴, R. Pogni⁴, T. Martellini³, A. Cincinelli³, A. Ugolini¹, D. Cavalieri^{1,5}¹University of Florence, Department of Biology, Sesto Fiorentino, Italy²Institute of Agricultural Biology and Biotechnology (IBBA), National Research Council (CNR), Pisa, Italy³University of Florence, Dept. of Chemistry "Ugo Schiff", Sesto Fiorentino, Italy⁴University of Siena, Department of Biotechnology, Chemistry and Pharmacy, Italy⁵CIB-Interuniversity Consortium for Biotechnologies, Trieste, Italy

Marine supralittoral environments are threatened by the accumulation of plastics and bioplastics transported by marine currents, winds, and local hydrodynamics. Bioplastics, such as starch-based polymers and chitosan–starch blends are gaining interest to reduce reliance on petroleum-based plastics, yet their environmental fate remains poorly understood. In this framework, the common sandhopper *Talitrus saltator*, key detritivore and scavenger species in supralittoral sandy beaches, was used to investigate bioplastic ingestion, survival, gut microbiota adaptation, and polymer modification after gut transit. To do this, adult *T. saltator* specimens were maintained under controlled conditions of photoperiod and temperature and fed exclusively with sheets of

two commercially available starch-based bioplastics or chitosan–starch blends (prepared with solvent-casting method); paper and dry fish food served as controls. All polymers were previously characterized by ATR-FTIR spectroscopy. Gut contents were sampled and gDNA was extracted and amplified for 16S rRNA (V3–V4) metagenomic sequencing on Illumina MiSeq Platform, and collected faecal pellets were also analysed by ATR-FTIR.

In summary, results showed an active consumption of polymers by supralittoral amphipods, with different effects on survival rate. Spectroscopic analyses revealed partial degradation of bioplastic during gut transit, indicating a potential digestive processing. Metagenomic profiling demonstrated an adaptation of gut microbial communities to bioplastic feeding, highlighting a positive selection of microbial genera associated to bioplastic degradation, suggesting a role in the partial digestion of polymers.

Overall, our results highlight the involvement of sandhopper-associated gut microbiota in bioplastic modification, opening to potential implication of these microbial consortia in bioplastic management.

B10	Plant Growth-Promoting Rhizobacterial Consortium from the Rhizosphere of <i>Vitis vinifera</i> cv. Falanghina: A Terroir-Oriented Approach to Sustainable Agricultural Biocontrol
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The request for increasing agricultural yields due to enhanced pressure on food production has inevitably led to the indiscriminate use of chemical fertilizers and other agrochemicals. Given the need for alternative approaches with less adverse environmental impacts, scientific interest has shifted to biological fertilizers and biocontrol agents, such as plant growth promoting rhizobacteria (PGPRs). In this contest, the aim of this study was the characterization of PGPR isolates from the rhizosphere of *Vitis vinifera* L. cv Falanghina for their direct and indirect plant growth promotion (PGP) capabilities. A total of 22 rhizobacteria were isolated from vineyards in Guardia Sanframondi (BN), south of Italy, and screened for *in vitro* ammonia, siderophore and indol-3-acetic acid (IAA) production and for phosphate solubilization. In addition, antimicrobial assays were performed to evaluate the antagonistic activity of each isolate against two key grapevine phytopathogens, *Rhizobium radiobacter* and *Botrytis cinerea*. Notably, the *Bacillus amyloliquefaciens* isolate generated the highest levels of ammonia, whereas *Pseudomonas fluorescens* and *P. aeruginosa* demonstrated the best siderophore production and phosphate solubilization abilities. Among all tested PGPR isolates, only *B. subtilis* and *B. cereus* synthesized significant amounts of indole-3-acetic acid (IAA). Moreover, *B. subtilis* and *B. amyloliquefaciens* strains distinguished for antibacterial and antifungal effects, respectively, against grapevine pathogens. Obtained results showed that the isolated rhizobacteria exhibit a broad spectrum of PGP traits, with nine strains in particular standing out as promising candidates for assembling a terroir-specific microbial consortium, offering an eco-friendly strategy to support sustainable viticulture across the entire wine production chain.

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Session C - Interactions between microbes/viruses and their hosts

Chairs: **F. Iannelli, R. Provvedi**

C1 First insights into bacterial and microalgal endosymbiont communities of various coral morphotypes from Maldives

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The Maldivian Archipelago is home to valuable coral reefs that have been extensively studied for their ecological diversity. However, the diversity of the microbiome in Maldivian corals remains largely unexplored. In this study, the microbiota compositions (including both algal endosymbionts and bacteria) were investigated for the first time across various coral morphotypes sampled in May 2022 from four Maldivian atolls (Ari, North Malé, South Malé, and Rasdhoo). Coral and gorgonian specimens were collected via scuba diving at reef sites located on both ocean-exposed reefs and lagoon sites, across various depths (0–40 m). Surface seawater samples were also collected near coral assemblages. Metabarcoding analyses were performed, targeting the 16S rRNA gene to assess bacterial composition, and the Internal Transcribed Spacer 2 (ITS2) rRNA region to evaluate microalgal endosymbiont diversity. Generally, the bacterial communities associated with corals exhibited significant diversity, which was primarily influenced by coral morphotype rather than depth or geographic location. These communities were also markedly different from those found in seawater. The three most abundant bacterial taxa in coral samples were Proteobacteria (ranging from 10 to 95%), Bacillota (formerly known as Firmicutes, ranging from 5 to 10%), and Planctomycetota (ranging from <1–30%). Most Symbiodiniaceae belonged to the genera *Cladocopium*-C and *Durusdinium*-D (>90%), while host specificity was observed for variant types. Overall, this study provides first insights into the structure of Maldivian coral microbiota, which could be crucial for monitoring the health of local coral populations and predicting the potential impacts of changing environmental conditions in the region.

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C2	Pisa 4: <i>in vitro</i> characterization and genetic engineering of a novel mycobacteriophage for therapeutic use
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Pathogenic mycobacteria pose a major clinical challenge due to intrinsic antibiotic resistance and intracellular persistence. Bacteriophages represent a promising alternative for treating such infections. This study aimed to genetically and phenotypically characterize Pisa 4, a novel mycobacteriophage isolated using *Mycobacterium smegmatis* mc²155 as host.

Whole-genome sequencing and bioinformatic analyses were performed to assess gene content. Phenotypic characterization included plaque assays and *in vitro* infection models using THP-1 macrophages and primary human neutrophils, analysed by confocal laser scanning microscopy. Phage engineering was performed *via* bacteriophage recombineering with electroporated DNA. Genomic analysis revealed that Pisa 4 lacks known virulence and antibiotic resistance genes and does not carry an integrase gene, suggesting a lytic profile. However, the presence of a transcriptional repressor suggests that the phage may exhibit temperate behaviour. Pisa 4 showed rapid replication (30-min latent period), a high burst size (100–250 PFU/cell) and stability at pH 4–6, consistent with phagosomal environments. Pre-infection treatment of *M. smegmatis* with Pisa 4 significantly reduced intracellular CFUs in THP-1 cells and increased intracellular PFUs, with confocal imaging confirming phage entry and co-localization with bacteria. Post-infection treatment was ineffective, likely due to a limited access to internalized bacteria. Notably, Pisa 4 did not trigger neutrophil extracellular trap release, supporting a favourable safety profile. Activity against *Mycobacterium abscessus* clinical isolates was observed in 4/49 strains, consistent with the narrow host range of mycobacteriophages. To enhance therapeutic potential, the repressor gene was deleted. These findings support Pisa 4 as a promising candidate against susceptible mycobacteria, with ongoing studies exploring engineered phage efficacy and host immune responses.

C3	Control of <i>Staphylococcus aureus</i> infection in burn wounds by physical plasma
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Wound infection by bacteria such as *Staphylococcus aureus* is one of the main causes of death in burn victims. The ability of *S. aureus* to form biofilms and develop resistance to first-line antibiotics presents a critical challenge, highlighting the need for alternative antibacterial strategies. Cold

atmospheric plasma (CAP) has emerged as a promising non-antibiotic approach with demonstrated antimicrobial efficacy, yet its mechanisms of action remain incompletely understood.

We investigated the therapeutic potential of CAP in a murine model of *S. aureus*-infected burn wounds. CAP treatment significantly accelerated wound healing, as shown by enhanced epidermal re-epithelialisation and extracellular matrix deposition during the tissue remodelling phase. These effects correlated with a notable reduction in bacterial burden.

Using an *in vitro* infection model, we uncovered that CAP stimulates host immune defences, promoting bacterial clearance without impairing immune cell proliferation. At the molecular level, we identify CAP-driven induction of toxic molecules contributing to bacterial elimination. Furthermore, we investigated the antibiofilm activity of CAP, combining quantitative and qualitative approaches.

Our findings provide mechanistic insights into the dual role of CAP in promoting tissue repair and in controlling staphylococcal skin infections. This work may open novel therapeutic perspectives for the treatment of multidrug-resistant pathogenic bacteria.

C4 Unraveling the microbial and immune landscape of Prostate Cancer

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Prostate cancer (PCa) is the most common malignancy among men and the fifth leading cause of cancer-related death. This study investigates the contribution of sexually transmitted infections (STIs), human endogenous retroviruses (HERVs), and immune dysregulation in prostate carcinogenesis. A total of 244 patients diagnosed with PCa, precancerous lesions (Borderline), benign prostatic hyperplasia (BPH) were enrolled. DNA extracted from urine samples was screened for a panel of STIs using multiplex RT-PCR. Humoral immune response against *Trichomonas vaginalis*, *Mycoplasma hominis*, HERV-K, HERV-H, and interferon regulatory factor 5 (IRF5) were assessed by ELISA, compared to age-matched healthy controls (HCs). A higher prevalence of STIs was observed in PCa and Borderline, with *M.hominis* being the most frequently detected. Anti-*M.hominis* antibody levels were significantly elevated in Borderline (86.96%), PCa (64.93%), and BPH (50.57%) versus HCs (28.70%), with similar trends for anti-*T.vaginalis* antibodies. No significant response was observed against HERV-K, HERV-H. Notably, anti-IRF5 antibody levels were reduced in PCa (32.09%), BPH (22.99%), Borderline (13.04%), as compared to HCs (67.83%).

These findings suggest a multifactorial model for prostate cancer involving chronic infections, immune modulation and HERVs elements. The elevated seropositivity rates for *M.hominis* and *T.vaginalis* in patients with PCa and precancerous lesions point to their potential role in establishing a pro-inflammatory microenvironment that may promote tumour initiation and progression. The marked reduction of anti-IRF5 antibodies in patients indicate possible immune suppression or dysregulation, consistent with IRF5's putative tumour-suppressor function in prostate cancer. These data underscore the importance of host-pathogen interactions and immune regulation in prostate carcinogenesis.

C5 Investigating Flavivirus Tropism and Entry Mechanisms in Human Cells

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Flaviviruses such as Dengue virus (DENV) and West Nile virus (WNV) are major human pathogens transmitted by arthropod vectors, responsible for millions of infections worldwide each year. Despite extensive research, the mechanisms by which these viruses enter human cells remain incompletely understood. Several host surface molecules, including C-type lectins that bind viral glycans and phosphatidylserine receptors that recognize the viral envelope, have been implicated in viral attachment. However, it remains unclear whether flaviviruses, like many other viruses, require specific entry receptors for productive infection. This study explores the hypothesis that flavivirus entry is mediated by specific receptors. Using a pseudovirus system based on WNV subgenomic replicons, we assessed the ability of envelope glycoproteins from all four DENV serotypes and WNV to mediate infection in a panel of human cell lines from diverse tissue origins. Differences in cellular permissiveness were observed, not only between DENV and WNV but also among DENV serotypes, suggesting the involvement of distinct host factors in viral entry. To identify these factors, we are conducting a genome-wide CRISPR-Cas9 knockout screen in highly permissive cell lines, alongside transcriptomic analyses to compare receptor gene expression across permissive and non-permissive cells. These complementary approaches aim to identify host surface proteins critical for flavivirus entry and to reveal the molecular determinants underlying flavivirus tropism, with potential implications for antiviral strategies.

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C6 Isolation Source Shapes the Cargo of *Saccharomyces cerevisiae* Extracellular VesiclesS. Nenciarini¹, F. Mensitieri^{2,6}, R. Alamanni³, M. Lulli⁴, N. Schiavone⁴, F.D. Piazz^{2,5}, P. Lionetti¹, D. Cavalieri³¹*Department of Neurofarba, University of Florence, Italy*²*Università degli Studi di Salerno, Dipartimento di Medicina, Chirurgia ed Odontoiatria "Scuola Medica Salernitana", Baronissi, Salerno, Italy*³*Department of Biology, University of Florence, Italy*⁴*Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Italy*⁵*Azienda Ospedaliera Universitaria "San Giovanni di Dio e Ruggi D'Aragona", Salerno, Italy*⁶*Scuola di Specializzazione in Patologia Clinica e Biochimica Clinica, Università degli Studi di Salerno, Italy*

Saccharomyces cerevisiae is a versatile yeast known for its roles in fermentation and as a model in genetics and cell biology. Beyond wineries, it colonizes diverse niches such as fermented foods and animal guts, where it interacts with surrounding organisms. Among its interaction mechanisms, *S. cerevisiae* releases extracellular vesicles (EVs)—nanosized, membrane-bound particles that transport proteins, lipids, and nucleic acids, mediating intercellular communication. While EVs are well-characterized in pathogenic fungi, little is known about their composition and function in environmental and industrial yeasts. To explore whether adaptation to specific habitats involves changes in EV cargo, we previously showed that EVs from yeasts isolated from milk-based fermented foods and grapes carry niche-specific immunomodulatory signals. These differences were driven more by ecological origin than by species identity.

Building on these findings, we conducted a proteomic analysis of EVs from twelve *S. cerevisiae*

strains isolated from fermented foods, grapes, human gut, and wasp gut. EV cargo clustered according to isolation source, with close similarity between the wasp gut and the grape strains, supporting the role of social insects as yeast reservoirs. Proteins enriched in gut-associated EVs were linked to adhesion and oxidative stress, while grape- and wasp-derived EVs favored metabolic processes like amino acid catabolism.

These results suggest that EVs reflect environmental adaptation and may play an active role in shaping both microbial communities and the communication with the host. By mediating communication with co-occurring organisms, EVs could promote cooperative behaviors, facilitating *S. cerevisiae*'s evolutionary success in specific environments.

C7	Enhanced clearance of phage-resistant <i>Klebsiella pneumoniae</i> strains by the innate immune system
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Phage therapy has emerged as a promising method to target antibiotic-resistant bacterial strains. Despite its potential, phage therapy has many challenges, including the development of phage resistance whose role in the context of pathogen virulence needs to be fully elucidated.

In this study, the interactions between phage-resistant *Klebsiella pneumoniae* strains and the innate immune system were evaluated. We generated a first-generation phage-resistant mutant, BO-FR-1, using the parental KKBO-1 clinical isolate (Sequence Type 258). Further, starting from BO-FR-1 strain, we isolated three second-generation phage-resistant mutants by using a different phage: FR-GP7-4, FR-GP7-7, and FR-GP7-10. Parental as well as mutant bacterial strains were sequenced and subjected to bioinformatic analysis. Results show the presence of mutations in phage resistant mutants leading to capsule depletion and lipopolysaccharide alteration.

Subsequently, human macrophages were infected with these five *Klebsiella pneumoniae* strains and differences between the parental strain and first- and second-generation phage-resistant mutants were analysed in terms of internalization index and intracellular bacterial killing capability by CFU assay. Additionally, we assessed each strain's susceptibility to the complement system by serum bactericidal assay. Results indicate that BO-FR-1 strain is more susceptible to phagocytosis, intracellular killing and complement system compared to KKBO-1. Conversely, FR-GP7-4, FR-GP7-7, and FR-GP7-10, although seem to better evade phagocytosis by macrophages compared to BO-FR-1 strain, are more effectively killed by the complement system.

Altogether, these results support the hypothesis that in the context of the interplay among phage, bacterial pathogen and host, the emergence of phage resistance may be beneficial for the host.

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C8	Emricasan (IDN-6556) inhibition of caspase-3/gasdermin-E pyroptosis pathway during <i>Neisseria meningitidis</i> infection could block bacterial epithelial barrier crossing
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Neisseria meningitidis (the meningococcus) is a Gram-negative bacterium that transiently colonizes the human nasopharynx. For poorly understood reasons, in some healthy carriers it causes Invasive Meningococcal Disease (IMD). A necessary condition for the bacterium to cause the disease is the crossing of the nasopharyngeal epithelial barrier. The latter comprises tight junctions that normally restrict pathogens. We found that the canonical inflammasome and the caspase-3/gasdermin-E pathway are activated in epithelium formed by Calu-3 cells when infected with *N. meningitidis* 93/4286 serogroup C strain, while the levels of the tight junction protein occludin are strongly reduced. Moreover, many genes under the control of NF- κ B were upregulated upon infection, among which the gene for the chemokine CCL3, known to damage tight junctions. The treatment with Emricasan (IDN-6556), but not with Disulfiram or VX-765, fully prevented the reduction of occludin levels in infected cells. Nevertheless, the molecule had no impact on CCL3 expression or canonical inflammasome activation. On the contrary, caspase-3 and gasdermin-E activation were abolished in infected cells after treatment with Emricasan. In conclusion, our data indicate that the meningococcus-induced activation of the caspase-3/gasdermin-E pyroptosis pathway in upper airway epithelia is sufficient to decrease the tight junction protein occludin levels, possibly aiding the bacterium in the epithelial crossing. Additionally, Emricasan, a pan-caspase inhibitor proven to be safe in clinical trials for liver diseases, prevented pyroptosis activation and occludin decrease induced by *N. meningitidis*, identifying it as a promising adjuvant drug for IMD.

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C9	Granuloma-Like Structures in Tuberculosis: A Hybrid Experimental and Modeling Approach for Drug Testing
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Mycobacterium tuberculosis (*Mtb*) evades host immunity to establish infection, and its phenotypic plasticity remains poorly understood. Host-Directed Therapies (HDTs), which aim to alter host responses rather than directly target *Mtb*, lack a standardized assay. We address this gap by advancing Granuloma-Like Structures (GLS) as a robust platform for TB research and drug screening.

GLS are *in vitro* models that replicate early granuloma formation and host-pathogen interactions. Despite their physiological relevance, their use is hindered by variability and technical complexity.

We demonstrated that the standardization of this method could be achieved by integrating colony-forming unit (CFU) counts with microscopy-based GLS size quantification, enabling reproducibility and cross-study comparison.

Through mathematical modeling, we showed that drug efficacy correlates with GLS size distribution. Consequently, incorporating GLS size distribution into analysis allows us to obtain more accurate and comparable results.

To strengthen the experimental framework, we integrated *in vitro* GLS assays with *in-silico* Agent-Based Modeling (ABM) to refine experimental design and support the interpretation of results. Comparative analyses with traditional monolayer models reveal that GLS more effectively captures immune-mediated drug effects.

We evaluated the activity of the standard antitubercular drugs (rifampin, isoniazid, bedaquiline, etc.) across both models, revealing that early GLS facilitates *Mtb* growth, consistent with the hypothesis that granulomas initially support infection.

This integrative framework establishes GLS as a standardized tool for TB drug discovery and to deeply understand host-pathogen interaction.

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853989.

C10 Unveiling the role of peroxisomes in SARS-CoV-2 Pathogenesis

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Peroxisomes are ubiquitous cellular organelles that contribute to key biological processes, including fatty acid β -oxidation, the synthesis of ether-linked glycerophospholipids, as well as the maintenance of the redox balance in the cell.

Previously, we found that peroxisomal dynamics are deeply affected during SARS-CoV-2 infection, suggesting a role for these organelles in SARS-CoV-2 pathogenesis. In the presence of SARS-CoV-2 infection, peroxisomes undergo significant changes, accumulating in the perinuclear area near the double-membrane vesicles (DMVs), the replication organelles where the replication of the viral genome takes place. The biological implications of these alterations and the precise contribution of peroxisomes to SARS-CoV-2 replication remain to be fully elucidated.

Here, we combined a high-throughput image-based screening with virological assays to understand if and how peroxisomes contribute to SARS-CoV-2 pathogenesis and spread. We identified PEX1, PEX6 and PEX13 peroxins as required for SARS-CoV-2 infection. Silencing of these peroxisomal factors significantly reduced the infectivity of the virus, while reintroduction of PEX6 alone was sufficient to restore the infection levels. These peroxins are crucial components of the peroxisomal matrix proteins import machinery, suggesting their involvement in recruiting peroxisomal enzymes necessary for viral infection.

In line with this, our findings revealed that both ether glycerophospholipid biosynthesis and the maintenance of cellular redox homeostasis are essential for SARS-CoV-2 infection.

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C11

Biogeography of the infant gut microbiota and identification of key microbial members

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During the first year after birth, the infant gut microbiome undergoes a rapid and profound compositional and functional transformation, impelled by an intricate network of intrinsic and extrinsic factors. This process results in increased taxonomic and functional diversification, alongside greater interindividual variability. To better understand this early-life ecosystem, this study assessed the interindividual variability of the infant gut microbiome using a comprehensive infant gut microbiome database of 5,288 fecal metagenomic data from healthy, full-term infants across various geographic locations. Our study identified six reference microbial communities, termed Early-Life Community State Types (ELi-CSTs), which not only capture specific compositional profiles and heterogeneity of the infant gut microbiome, but also record the extensive transformation experienced by this developing microbial community during the first year of human life. Indicative Species analysis and Random Forest modeling assisted the precise identification of unique, key taxonomic signatures that are critical to the structure of each ELi-CST, highlighting microbial taxa with pivotal roles in shaping the infant gut microbiota. To complement these findings, we established a bacterial biobank comprising 182 genome-sequenced isolates corresponding to key taxa involved in early life gut microbiota assembly. This biobank enabled *in vitro* investigations into microbial cross-talk among key modulators, offering novel insights into the molecular interactions and cooperative dynamics behind early microbiome development.

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C12

Loss of FadD reduces *Shigella flexneri* invasiveness by impairing of the Type III Secretion System

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Shigella spp. is a Gram-negative bacterium that causes shigellosis, an acute intestinal infection due to its ability to invade and damage colonic epithelial cells. In this study, we demonstrated that the *fadD* gene, which encodes an acyl-CoA synthetase involved in β -oxidation of fatty acids, influences the infectious capacity of *Shigella flexneri*. Treatment with exogenous fatty acids is known to interfere with VirF, the master regulator of virulence, leading to reduced expression of *virB*, a transcriptional activator essential for inducing the type III secretion system (T3SS), which is required for host cell invasion. Our results show that the loss of *fadD* impairs VirF activity, resulting in significantly reduced *virB* expression at both the transcriptional and post-transcriptional levels. Furthermore, we demonstrated that the Δ *fadD* mutant exhibits a markedly reduced ability to invade epithelial cells, likely due to impaired T3SS expression. In macrophages,

the mutant also shows reduced survival during the early stages of infection, presumably due to a defect in escaping the phagocytic vacuole. We hypothesize that in *ΔfadD* strains, the absence of the enzyme disrupts normal β-oxidation, leading to intracellular accumulation of endogenous fatty acids that may interfere with VirF activity and inhibit its function. Overall, these findings reveal a direct link between fatty acid metabolism and the regulation of *Shigella* virulence, highlighting the critical role of *fadD*. This mechanism offers new insights into host–pathogen interactions and suggests potential targets for therapeutic intervention aimed at modulating bacterial virulence through metabolic pathways.

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POSTER SESSIONS

Session A - Microbial genetics and genomics

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A13 *cps* and *hrp*: two mobilizable *Neisseria* loci implicated in invasive disease

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There is growing evidence of invasive infections caused by *Neisseria* spp. other than *N. meningitidis* and *N. gonorrhoeae*. A wide range of systemic infections have been attributed to *N. elongata*, *N. mucosa*, *N. sicca*, *N. subflava*, and *N. cinerea*, particularly in immunocompromised individuals or after surgery and, in some cases, in healthy individuals. Recently, two human cases of infection have been attributed to a new *Neisseria* species, *N. brasiliensis*, of one which involved bacteremia. Genomic analysis showed that this bacterium is closely related to *N. iguana*, but contains a capsule biosynthetic locus (*cps*) similar to that of *N. meningitidis*. Here, we show that the *hrp* locus, which is absent in *N. gonorrhoeae* and in non-pathogenic *Neisseria* spp., is also present in *N. brasiliensis*. The *hrp* locus encodes the two-partner secretion system HrpB/HrpA, which is involved in critical steps of meningococcal cell infection: escape of bacteria from the internalization vacuole, interaction with dynein, induction of pyroptosis, and inhibition of apoptosis. Because phylogenetic analysis with vertically transmitted genes (selected for their conservation and low recombination rate) clearly demonstrates the long phylogenetic distance between *N. brasiliensis* and *N. meningitidis*, the presence of similar *cps* and *hrp* loci in these two unrelated species suggests that these loci specifically contributed to the evolution of the pathogenic phenotype in *Neisseria*. This belief is supported by evidence that meningococcal isolates from healthy carriers often exhibit rearrangements of these loci leading to loss-of-function or, in the case of *cps*, capsular switching or capsule replacement with teichoic acid-like structures.

A14 Ploidy-driven genomic recircuiting in prokaryotes linked to bacterial persistence

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Bacterial persistence, a cunning survival tactic marked by a phenotypic transformation, allows genetically identical cells to defy the onslaught of antimicrobials. Research unveils that ploidy – number of chromosome copies nestled within a cell - plays a pivotal role in reshaping this biological landscape. We meticulously examined the profound connection between ploidy variation and persistence in *Escherichia coli*, employing a multidisciplinary approach that integrated single-cell ploidy measurement, flow cytometry, Oligopaint DNA FISH, and -omics analyses. Flow cytometry data revealed growth phase-dependent DNA content distributions, with rapidly dividing populations exhibiting multimodal profiles indicative of subpopulations differing in chromosomal content. Using Oligopaint-based hybridization to target ori- and ter-proximal loci, we visualized these replication patterns, successfully resolving origin signals and partially resolving terminus regions. This suggests that ploidy heterogeneity may reflect underlying metabolic states relevant to persistence. Building on these results, our next objective is to sort bacterial subpopulations based on their ploidy profiles using FACS and perform antibiotic persistence assays on each fraction. This will allow us to directly quantify the persistence potential associated with distinct ploidy states. Following this, we will apply RNA sequencing to these sorted subpopulations to uncover differentially expressed genes and reconstruct transcriptome-based cell cycle parameters. This will enable us to assess how gene expression patterns and cell cycle timing correlate with ploidy and persistence capacity. By linking ploidy to gene regulation and persistence, this project aims to enable new strategies to predict and counter persistence, supporting personalized antimicrobial therapies.

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A15 CodY and RelA Coordinate Expression of *aprE*, a Key Protease Gene with Industrial Applications, in *Bacillus subtilis*

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Bacillus subtilis adapts to nutrient limitation through a complex regulatory network involving the stringent response and the global transcriptional regulator CodY. Under amino acid starvation, (p) ppGpp alarmones - synthesized by RelA, YjbM, and YwaC - accumulate and reduce intracellular GTP levels. Since GTP is essential for CodY activity, its depletion leads to derepression of numerous stationary-phase genes previously under CodY-mediated repression.

Among these is *aprE*, which encodes a major extracellular alkaline protease expressed upon entry into stationary phase. We previously demonstrated that CodY represses *aprE* both directly, by binding near its transcription start site, and indirectly, by repressing *scoC*, another negative regulator of *aprE*.

Here, we examined how activation of the stringent response affects *aprE* expression and explored the interplay between CodY and the primary stringent response regulator, RelA, in its regulation. Using a *PaprE-lacZ* transcriptional fusion, we observed that deletion of either *relA* or *codY* reduced *aprE* expression during growth in sporulation medium. Unexpectedly, the *relA codY* double mutant exhibited significantly higher expression than the wild type, with overexpression detectable even

during exponential phase, when *aprE* is typically repressed by multiple transcription factors. Given the importance of transcription start site (+1 position) identity in modulating gene expression during the stringent response in *Bacillus subtilis*, we investigated the effect of mutating the native +1A of *aprE* to G on its expression.

These findings enhance our understanding of transcriptional regulation under nutrient stress and are relevant to industrial biotechnology applications involving microbial protease production, such as subtilisin (AprE).

A16

Metagenomic analysis reveals functional traits of *Rubrobacter* species involved in the rosy discoloration of church frescoes in Georgia

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Microbial biodeterioration, such as the formation of pink biofilms that cause rosy discoloration on stone monuments and mural paintings, presents significant challenges for preserving cultural heritage worldwide. Global warming, which exacerbates this issue, may promote the growth of xerophilic and xero-tolerant microbial communities. However, the mechanisms by which microbial species colonize pink biofilms on artworks remain unclear. In this study, a multidisciplinary approach combining physicochemical analysis, scanning electron microscopy, and next-generation sequencing was applied to samples from two frescoed churches in Georgia: Gelati Cathedral and Martvili Church. The samples were predominantly colonized by *Actinomycetota* members, and metagenomic data revealed several *Rubrobacter* metagenome-assembled genomes (MAGs), including *Rubrobacter aplysinae*, from the pink biofilms at Gelati. Genomic analysis indicated the presence of genes involved in carotenoid biosynthesis, suggesting a cooperative bacterial mechanism via genetic complementation that may contribute to the formation of pink biofilms. Additionally, several MAGs contained putative biosynthetic gene clusters, drug resistance genes, and genes encoding tolerance to quaternary ammonium compounds, which are commonly used in biocides, exerting selective pressure on microbial communities within the biofilms. This study offers the first genomic evidence linking heavy contamination by *Rubrobacter*-related bacterioruberin-producing bacteria to the rosy discoloration of fresco paintings.

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A17	Toward the Genomic Characterization of the Enigmatic ‘<i>Candidatus Saccharimonadia</i>’ through Targeted Enrichment from Human Saliva
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“*Candidatus Saccharimonadia*” is a low-abundance bacterial lineage in human oral microbiota. Recent evidence revealed the bacterium has immuno-modulating effects and it has been found increased in patients with inflammatory conditions, such as periodontitis, inflammatory bowel disease and food allergy. This lineage may also act as a keystone species, reshaping microbial community networks through its presence by modulating phage predation of host infection. Studying these fastidious bacteria has been challenging due to their small cell-sized, reduced genomes and epibiotic lifestyle, which complicated cultivation and genomic characterization. Current genomic approaches based on co-cultivation and shotgun metagenomics, are limited by cost and complexity. A reliable and accessible protocol to enable the genomic characterization of this group is needed. We developed a cost-effective “*Ca. Saccharimonadia*” enrichment protocol (SE), that when applied to fresh human saliva samples, increases the abundance of its DNA from ~5% to ~80%, while reducing human reads to less than 10%. Thus, high quality genome assemblies were obtained, with statistics comparable or better than “*Ca. Saccharibacteria*” genomes available in public databases. The SE protocol represents an advancement in studying “*Ca. Saccharimonadia*” offering a direct genomic access to ultra-fastidious members of the human microbiota. By enabling efficient genome sequencing, it opens new perspectives for exploring the role of these bacteria in the oral microbiota and their involvement in inflammatory and immune-mediated diseases as well as microbial-host interactions. Moreover, this methodology requires only standard laboratory equipment commonly available in laboratories and significantly lowers the sequencing depth, which further reduces the computational resources.

A18	Gut Microbiota in Psoriatic Arthritis: A Comparison Between Remission and Active Disease
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Psoriatic arthritis (PsA) is a chronic inflammatory disease characterized by joint inflammation and skin lesions. Recent research has highlighted the critical role of the gut microbiota in the pathogenesis and progression of PsA. Thanks to a better understanding of PsA, new biotechnological drugs have been developed that are capable of improving the signs and symptoms of inflammation and inhibiting joint damage in peripheral joints, leading to an improvement in quality of life and functional status.

In this preliminary study, changes in the gut microbiota were evaluated in patients with active disease compared to patients in remission undergoing drugs treatment or in drug-free remission. Fecal samples were collected from eight patients with PsA and subjected to DNA extraction, high-throughput sequencing and metagenomic analyses, which revealed distinct microbial profiles associated with PsA.

Results highlighted differences in the gut microbiota of patients with active disease compared to those in remission either under biotechnological treatment or in drug-free remission. They indicate an alteration of various microbial groups in the active phase and a more or less evident trend of recovery in patients in remission, linked to the varying depth of remission due to the different mechanisms of drugs action.

These preliminary data suggest that we should monitor the composition of the gut microbiota over time in patients, to observe whether, in addition to suppressing inflammation at the joint and skin level and the disappearance of clinical signs of the disease, a normal microbiota is also restored.

A19

cgSNP-based genomic evolution of BSI-Carbapenem-Resistant *Klebsiella pneumoniae* carrying different carbapenemases and hyper-virulence genes

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Introduction: *Klebsiella pneumoniae* is an ESKAPE pathogen causing severe healthcare-associated infections with high mortality rates. Of particular concern is its increasing carbapenem-resistance, first-line antimicrobials in therapy, resulting in a clinical challenge related to the horizontal transfer of mobile genetic elements carrying carbapenemases.

We aimed to characterize 20 clinical Carbapenem-Resistant *K.pneumoniae* (CRKP), isolated from bloodstream-infections collected in South-Italy hospitals, by Genomics using Phylogenomics, Typing, Virulomics, and Resistomics performed by Illumina Whole-genome sequencing, core-genome SNP-clustering, and Pathogenwatch-typing.

Results: Data evidenced 3 main ST-307 core-genome ancestor phylogenetic lineages carrying *bla*_{KPC-3}. The ST-307 KL-102 gPhyl lineage-I was characterized by the *bla*_{KPC-3} and CTX-M-15 ESBL. The ST-307 KL-102 gPhyl lineage-II carried *bla*_{KPC-3} along with CTX-M-15 and TEM-1 ESBLs. The gPhyl lineage-III showed 2 clusters containing an ST-307 KL-102 gPhyl lineage-III having the *bla*_{KPC-3} and CTX-M-15 ESBL, and a second complex gPhyl lineage-IV evolving in two other sub-lineages. Sub-gPhyl lineage-IVa was characterized by the co-presence of different carbapenem-resistant genes, whereas the sub-gPhyl lineage-IVb was labeled by hypervirulence-related genes. The ST-101/3366 KL-17 sub-gPhyl lineage-IVa carried *bla*_{OXA-48} and *bla*_{NDM-1}, whilst the hypervirulent ST-147 KL-64 harboured one among the *bla*_{NDM-1} or *bla*_{NDM-3}, CTX-M-15 ESBL, and *armA*. This hypervirulent sub-lineage-IVb had a Virulence_score=4, due to the occurrence of aerobactin and yersiniabactin (siderophores) and hypermucoidy-responsible genes (*rpmA*).

Conclusion: Our data showed the ST-307 kL-102 CRKP core gPhyl-lineage-III carrying *bla*_{KPC-3} and CTX-M-15 ESBL as a high-risk ancestor lineage prone for a concomitant evolution towards sub-lineages harbouring combinations of plasmidic carbapenem-resistance (*bla*_{OXA-48} and *bla*_{NDM-1}) or NDM-carbapenemase genes (*bla*_{NDM-1} or *bla*_{NDM-3}) associated with high-virulence.

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A20 Artificial Intuition for Accelerating the Process of Antimicrobial Drug DiscoveryG. Stelitano¹, C. Bettoni¹, M. Cocorullo¹, J. Marczyk², L.R. Chiarelli^{1*}¹*Department of Biology and Biotechnology, University of Pavia, Italy*²*Ontonix s.r.l., Sondrio, Italy*

The development of new drugs is a challenging, expensive and time-consuming process. This is an especially important issue with regard to antimicrobials, as they are affected by the global problem of resistance developing and spreading. This highlights the importance of accelerating drug development processes while reducing costs. In this context, new bioinformatics tools can support drug development by limiting and shortening the in vitro evaluation of promising results, thereby minimising costs. Recently, new artificial intelligence (AI)-based tools have been developed for the de novo design of new molecules and the identification of inhibitory features of microbial compounds that are already available and active against *Mycobacterium tuberculosis*. The compounds were subjected to molecular dynamics simulation and the results were analysed using Quantitative Complexity Measurement (QCM) to determine their complexity profiles. Comparing different analogues within each series revealed a relationship between the complexity of the chemical structures and their importance for the biological activity of each compound. This suggests that QCM could be a useful tool for guiding the optimisation process. The first application of this technology in drug development produced interesting results, indicating that the Quantitative Complexity Theory (QCT) method incorporated in AI4 could be used for rational drug design in the near future.

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A21 Monitoring marine microbial biodiversity in the Mediterranean: the Italian omics observatory networkM. Coci^{1,5}, G.M. Quero^{1,5}, M. Celussi^{2,5}, V. Manna^{2,5}, E. Banchi^{2,5}, D. Sarno^{3,5}, R. Piredda^{3,5}, S. Magozzi^{4,5}, M. Castellano^{4,5}, F. Massa^{4,5}, C. Oliveri^{4,5}, M. Pinat^{1,5}, L. Vezzulli^{4,5}, G.M. Luna^{1,5}¹*National Research Council (CNR), Institute for Biological Resources and Marine Biotechnologies (IRBIM), Ancona, Italy*²*National Institute of Oceanography and Applied Geophysics - OGS, Trieste, Italy*³*Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy*⁴*Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV), University of Genoa, Italy*⁵*National Biodiversity Future Center (NBFC), Italy*

The Italian Omics Observatory Network was established within Activity 5 of Spoke 2 of the National Biodiversity Future Center. Its primary goal is to strengthen a collaborative network among four Italian Long-Term Ecological Research (LTER) sites and to introduce omics analyses through harmonized protocols, adding the omic data to existing physical, chemical and biological datasets. This initiative aligns with global efforts for microbial biodiversity monitoring in the marine environment. After one and a half years, we can account for 12 sampling campaigns conducted across the 4 stations (Senigallia, Portofino, Golfo di Trieste, Golfo di Napoli). These campaigns have resulted in the establishment of associated biobanks, containing size-fractionated seawater filters from both surface and subsurface layers. DNA extracted from these fractions will undergo sequencing for 16S and 18S rRNA gene and ITS metabarcoding, as well as metagenomics. In parallel, zooplankton and phytoplankton samples have been collected for both microscopic observation and metabarcoding analysis. These comprehensive datasets, along with associated

metadata, will be made available to the scientific community through a single data platform. This strategic scientific infrastructure not only serves as a repository of essential biological raw materials but also aims to accelerate discovery and innovation in the marine field, providing crucial data for biodiversity monitoring and conservation.

A22

Mycobacterium tuberculosis and Human Methionine aminopeptidase comparison, production and activity set up to develop novel selective antitubercular inhibitors

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is a major global health challenge in particular due to Multi-Drug Resistant (MDR) strains, and extensively Drug Resistant (XDR) strains. Methionine aminopeptidase (MetAP) is essential for the *N*-formylmethionine excision of peptides during protein synthesis, hence regulates proteins activity by determining and enabling additional modifications. MetAPs are indispensable for all life forms, including eukaryotes and prokaryotes and is particularly vital for the proliferation of bacteria. Consequently, MetAP is a potential target for the development of antibacterial and anti-tuberculosis therapies. In Mtb, the *mapB* gene encodes the MetAP1c isoform that is of major significance.

Through an *in-silico* screening we created a library of potential MapB inhibitors, characterized by bipyridine, picolinate, and pyrrolo-pyridine scaffolds. These compounds were assayed against the Mtb MapB, produced in recombinant form in *E. coli*, affording several of active compounds, and demonstrating concrete potential for further studies aimed at their optimization. However, for an inhibitor to be considered a valid drug candidate, it is essential to evaluate its selectivity to avoid potential toxic effects due to inhibition of the homologous human enzyme. Therefore, we assessed the biochemical activity and inhibitory effect of the selected compounds on also against the human counterpart HuMetAP1, identifying molecules highly selective for the Mtb enzyme. Overall, these results represent a significant step forward in the development of new, targeted, and less toxic drugs, contributing to the search for innovative strategies against tuberculosis.

A23

A 69.9-kbp long inverted repeat increases genome instability in a strain of *Lactobacillus crispatus*

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Long inverted repeats (LIRs) of DNA sequences longer than 30 kbp are rare in prokaryotes. Here, we identified two 69.9-kbp LIRs in the genome of *Lactobacillus crispatus* M247_Siena, a derivative of probiotic strain M247. Complete genome sequence of M247_Siena was determined using Nanopore and Illumina technologies, while genome structure was analyzed using ultra-long (>80 kb) Nanopore read mapping and PCR. Compared to parental M247 strain, M247_Siena genome contained an additional copy of a 69.9-kbp long chromosomal segment, replacing a 15.4-kbp DNA segment. The duplication resulted in two copies of the 69.9-kbp long chromosomal segment, arranged as LIRs located 224.4-kbp apart upstream and downstream the chromosomal origin of replication, respectively. Both 69.9- and 15.4-kbp segments were delimited by the same

insertion sequences (IS1201 and ISLcr2) and PCR analysis of the M247 population revealed low rates (~1.28 per 10⁴ chromosomes) of chromosomal rearrangements involving these regions. In contrast, quantitative analysis of chromosomal rearrangements using Nanopore reads, indicated that the 69.9-kbp LIRs in M247_Siena increased genomic instability, as evidenced by two alternative chromosomal structures detected at frequencies of 23.3% and 76.7% (~1 out of 5 chromosomes). Comparative analysis of *L. crispatus* complete genomes revealed no LIRs similar to those of M247_Siena supporting the hypothesis of a laboratory-based evolution of the M247 strain, which led to its derivative M247_Siena. Furthermore, long repeats of other DNA segments and chromosomal rearrangements, mostly associated to insertion sequences, were detected in 8 and 9 out of 25 *L. crispatus* genomes, respectively, highlighting genomic instability as a trait of the species.

A24 *arn*-dependent colistin resistance in *Pseudomonas aeruginosa* biofilms

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Bacteria within biofilms appear more tolerant to antibiotics, including colistin, which is considered the last-resort antibiotic against multidrug resistant Gram-negative bacteria (doi:10.1189/fcimb.2022.851784). Colistin targets the bacterial outer membrane (OM), by electrostatic interactions, leading outer and inner membranes permeabilization, cytoplasm leakage and cells death. However, OM remodeling makes cells resistant to colistin, which in *P. aeruginosa* is mainly due to lipid A aminoarabinylation mediated by the *arn* operon. It has previously been shown that extracellular DNA makes *P. aeruginosa* biofilms tolerant to colistin, possibly due to the *arn* operon (doi: 10.1371/journal.ppat.1000213). Thus, we have analyzed *arn* expression and colistin susceptibility in *P. aeruginosa* biofilms (MBIC, Minimal Biofilm Inhibition Concentration) of reference strains and cystic fibrosis clinical isolates, both *wt* and Darn mutants. Finally, colistin susceptibility was modulated by inhibitors of ArnT, the last committed enzyme of the lipid A modification.

Our results showed higher *arnT* expression in biofilms as compared to planktonic cells, in agreement with higher biofilm resistance to colistin. Additionally, colistin resistance was lower in biofilms of Darn mutants respect to the parental strains. Also, the ArnT inhibitors FDO and FDO-H (doi:10.1093/jac/dkaa200; 10.1021/acs.joc.0c01459) potentiated the activity of colistin against *P. aeruginosa* biofilms, with MBIC reduction ranging from 128- to 8-fold in clinical isolates. Of note, similar results were obtained with *Klebsiella pneumoniae* clinical isolates. Collectively, these results support the use of ArnT inhibitors to potentiate colistin activity in those clinical settings in which biofilm-grown cells are prevalent, such as chronic infections.

A25

Cobalt as a Backup: How a Metal Switch Can Sustain *Pseudomonas aeruginosa* Virulence under Zinc StarvationC. Demingo^{1,2}, E. Michetti¹, V. Secli¹, M.L. Astolfi³, F. Pacello¹, A. Battistoni¹, S. Ammendola¹¹Department of Biology, Tor Vergata University of Rome, Italy²PhD Program in Cellular and Molecular Biology, Tor Vergata University of Rome, Italy³Department of Chemistry, Sapienza University of Rome, Italy

Bacterial pathogens must rapidly adapt to fluctuating metal availability within the host, where essential micronutrients are actively sequestered as part of nutritional immunity. Among these, zinc is a critical cofactor for a wide array of enzymes and regulatory proteins, and its availability is tightly linked to the expression of key virulence traits in *Pseudomonas aeruginosa*. This opportunistic pathogen employs different zinc acquisition systems transcriptionally regulated by the Zinc Uptake Regulator Zur, enabling its persistence within the host. Recently, Zur-controlled operons involved in the uptake/export of cobalt have been identified. Although cobalt is primarily associated with cobalamin-dependent reactions, its selective import under zinc-limiting conditions suggests a potential role for cobalt in bacterial adaptation to zinc scarcity. Yet, the functional relevance of this metal-based compensation remains poorly defined.

This study shows that cobalt supplementation alleviates key effects of severe zinc deficiency in *P. aeruginosa*, including reduced pyocyanin production, impaired swarming motility, and enhanced sensitivity to oxidative stress. Furthermore, *in vitro* assays demonstrate that cobalt can functionally replace zinc in the proteases LasA and LasB and the transcriptional regulator Zur. Finally, we found that a *P. aeruginosa* strain deficient in the pyochelin-cobalt receptor PA2911 exhibits impaired colonization of *Galleria mellonella* larvae, supporting the hypothesis that cobalt compensatory function may be crucial during infection. Our results suggest that cobalt may play a broader biological role than previously recognized, highlighting its potential to support *P. aeruginosa* survival and pathogenicity in zinc-limiting environments.

A26

AmpWrap: A One-Line Fully Automated Amplicon Metabarcoding 16S rRNA Gene AnalysisL. Doni^{1,2*}, A. Marotta¹, L. Vezzulli^{1,2}, E. Bosi^{1,2}¹Department of Earth, Environmental and Life Sciences (DISTAV), University of Genoa, Italy²NBFC, National Biodiversity Future Center, Palermo, Italy

The revolution of next-generation-sequencing has driven the establishment of metabarcoding as an efficient and cost-effective method for communities taxonomical profiling. Amplicon sequencing of taxonomic marker genes, such as the 16S rRNA gene in prokaryotes, provides a comprehensive census of the microbial community. The advent of long-read technologies made feasible to sequence the whole 16S rRNA gene rather than only a few regions, with the potential to achieve species-level resolution. Despite the affordability and scalability of such experiments, a major bottleneck remains the lack of integrated and user-friendly analytical workflows. Current pipelines often require the use of multiple tools with complex dependencies, and parameter optimization is frequently performed manually, limiting reproducibility and overall efficiency. To fill this gap we developed, AmpWrap, an automated, one line workflow designed to analyse both short and long reads, requiring minimal efforts by the user and automatically optimising the trimming parameter to retain the maximum number of reads and information while reducing noise.

A27	Cefiderocol activity against planktonic and biofilm forms of β-lactamase-producing <i>Pseudomonas aeruginosa</i> from people with cystic fibrosis
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Chronic *Pseudomonas aeruginosa* infections are a leading cause of acute pulmonary exacerbations in people with cystic fibrosis (pwCF). Intrinsic antibiotic resistance and biofilm formation complicate treatment. This study investigates the genomic diversity and cefiderocol efficacy against planktonic and biofilm-associated forms of *P. aeruginosa* isolates from pwCF. Eight *P. aeruginosa* clinical isolates and three laboratory strains underwent whole genome sequencing (WGS). Biofilm formation was assessed through biomass, cell count, metabolic activity, and extracellular DNA (eDNA). The minimum bactericidal concentration (MBC₉₀) and biofilm eradication concentration (MBEC₉₀) were also determined. WGS revealed significant genomic diversity, identifying ten distinct sequence types (STs). Antibiotic susceptibility testing (AST) showed that 10/11 strains were susceptible to cefiderocol, with one isolate (MPA9) displaying resistance linked to the *blaOXA486* gene. Adding the β -lactamase inhibitor avibactam (AVI) restored susceptibility in this resistant strain. Although iron metabolism genes were highly conserved across isolates, MPA9 lacked the *fpvA* iron receptor, potentially contributing to cefiderocol resistance. Biofilm formation significantly increased tolerance to cefiderocol, with an 8-fold rise in MBEC₉₀ compared to MBC₉₀. These findings highlight the genomic diversity and adaptive potential of *P. aeruginosa* in pwCF. Cefiderocol shows promise against planktonic and biofilm-associated *P. aeruginosa*, and combining it with AVI may counteract β -lactamase-mediated resistance.

A28	Beyond Resistome and Virulome: The PBPome as a Novel Phylogenetic Marker for Adaptive Evolution and Recurrent Infections in <i>Enterococcus faecalis</i> Infectious Endocarditis
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Infective endocarditis (IE) caused by *Enterococcus faecalis* represents a serious clinical challenge due to their intrinsic and acquired resistance mechanisms and remarkable ability to form biofilms. This study investigates a collection of 19 *E. faecalis* IE isolates, exhibiting varying degrees of biofilm formation and multifaceted antibiotic resistance, virulence and PBPs profiles.

| Abstracts |

Isolates exhibited 13 distinct multilocus sequence types (STs), the most frequent being ST102 and ST72 (3 strains each). Two ST102 isolates, among over 1600 housekeeping genes, showed the same cgMLST, and two strong biofilm producers, belonging to ST21, were associated with recurrent IE episodes, emphasizing the persistent and adaptable nature of these pathogens.

Six antimicrobial resistance determinants were always present [(MLS)*mph*(D); (MLS)*isaA*; *dfrA*; *efrA/B*; *emeA*]; resistance to aminoglycosides and tetracyclines was rarer.

Core virulence factors were surface proteins *fss1*, *ebp* locus, *efaA*, *elrA*, *srtA/C*; biofilm formation *bopD*; sex pheromones; thiol peroxidase *tpx* capsule biosynthesis *cpsA/B*.

All but 2 strains lacked the *cyl* operon, involved in acute rather than persistent infections.

Beyond conventional genotyping and resistome/virulome analysis, we introduce a novel concept for *E. faecalis* clustering, based on synchronous mutations within Penicillin-Binding Proteins (PBPs), hereby termed “PBPome”.

Our findings reveal that *E. faecalis* isolates exhibit a distinctive pattern of synchronous mutations in specific class A (1a, 1b) and B (2, 4) PBPs which consistently provide a unique molecular fingerprint. These fingerprints closely mirror the clustering observed with core genome analysis, suggesting co-evolutionary changes in these crucial enzymes, tightly linked to the overall genomic evolution and clonal expansion of these pathogens.

Acknowledgements

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A29

Unveiling microbial dynamics in short hospital stays: implications for human and hospital microbiomes

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AMR and ESKAPE pathogens global rise urges recognition of hospitals as key microbial transmission hubs. In this context, patients' short hospital stays (SHS) may represent critical windows for exchange, yet remain poorly characterized.

As part of the ANTHEM project (AdvaNced Technologies for Human-centrEd Medicine), funded under the National Complementary Investment Plan to NRRP, we conducted a cohort study in the pre-admission ward of Fondazione IRCCS San Gerardo dei Tintori Hospital (Monza, Italy), collecting 758 environmental and 260 skin microbiome samples from over 130 adult outpatients across four days. All samples underwent high-throughput DNA sequencing; here, we report a 196 samples focused analysis. Despite strong inter-individual variability in skin microbiota (PERMANOVA on Bray-Curtis distances, $p = 0.001$), microbial exchange between environment and outpatients occurred during SHS. A total of 856 ASVs were newly detected on post-visit outpatients' skin, 27.1% were also found in environmental samples, with the floor harboring the highest number (Kruskal-Wallis, $p = 1e-09$; post hoc Dunn, $p < 0.001$). The proportion of newly acquired ASVs on skin that overlapped with environmental ASVs collected the same day varied across outpatients (20-73%). All outpatients shared ASVs with others sampled the same day (28-80%), with *Methylobacterium*, *Sediminibacterium*, and *Streptococcus* among the most shared taxa. Environmental ASVs increased across time points, peaking during outpatient flow, with 3-5% matching pre-visit skin ASVs. Our findings show that SHS reshape hospital and human microbiomes through multidirectional exchange, with potential implications for microbial diversity and human health, and ultimately informing future infection prevention strategies.

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A30

Exploring the role of DNA methylation in a bacterial strain with biotechnological relevanceU. Genchi¹, A. Firrincieli², S. Di Silvestro¹, B. Barosa¹, D. Roncarati¹, R. Alduina³, M. Cappelletti¹¹Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Italy²Department for Innovation in Biological, Agro-Food and Forest Systems (DIBAF), University of Tuscia, Viterbo, Italy³Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy

Rhodococcus opacus PD630 is known for its ability to degrade hydrocarbon and aromatic compounds, including lignin-derived molecules, and to produce and accumulate lipids. To date, no study has investigated the role of DNA methylation in regulating these biotechnologically relevant processes. DNA methylation is carried out by DNA methyltransferases (MTases), enzymes that catalyze post-replicative modifications on DNA by transferring a methyl group to adenosine or cytosine bases.

In this study, we first characterized the genes present in a *Rhodococcus* genome, encoding methyltransferases involved in DNA methylation. In particular, we conducted an in-silico analysis to identify the DNA methyltransferase genes and we created the deletion mutants using a two-step recombination method. The results indicated the presence of both types of MTase, i.e. one belonging to Restriction-Modification (RM) systems and another being categorized as “orphan”. The phenotypes of the PD630 wild-type (WT) strain and the MTase mutant strains were analysed considering the growth behaviour under different conditions and the stress response. Methylomic analysis was also carried out using Oxford Nanopore sequencing technology. The sequencing data identified the methylation consensus sequence and revealed the differential methylation level between the mutants and the WT. Some of the differentially methylated promoters were located upstream of genes involved in lipid biosynthesis, hydrocarbon degradation and stress response suggesting the involvement of DNA methylation process in the regulation of genes with biotechnological relevance.

A31

Leveraging 4f-SAMMY-seq protocol in Prokaryotes as a high-resolution technology to study Nucleoid accessibilityU.M. Iannacchero^{1*}, E. Chiti^{2*}, V. Rosti^{1,3*}, A. Vannini², C. Lanzuolo^{1,3§}, D. Roncarati^{2§}, E.M. Pinatel^{1§}¹Institute of Biomedical Technologies, National Research Council, Segrate, Italy²Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Italy³INGM, National Institute of Molecular Genetics “Romeo ed Enrica Invernizzi”, Milan, Italy

Bacterial chromosome self-organizes into the nucleoid, a membrane-less region where Nucleoid Associated Proteins (NAPs) and RNAs regulate its accessibility. Bacterial HiC-based protocols provided insights into nucleoid organization; however, they fail to capture interactions below one kilobase, even pushing sequencing depth. We describe the first application on prokaryotes of 4f-SAMMY-seq (4-fraction Sequential Analysis of MacroMolecules Accessibility sequencing), a crosslink and restrictions enzymes-free technology conceived to study chromatin organization, which achieved HiC-level resolution at lower sequencing depth in eukaryotes.

We aim to adapt 4f-SAMMY-seq protocol and analysis tools to *Helicobacter pylori* in exponential growth phase, to investigate nucleoid organization at high-resolution.

4f-SAMMY-seq biochemically separates DNA and associated proteins or RNAs into four fractions according to their physico-chemical properties. Fractions are sequenced, and DNA accessibility is

assessed through fraction-pair relative ratios. Generally, accessible fractions correlate with ATAC-seq signals and open chromatin marks, while less accessible fractions with closed chromatin marks. We adopted the nf-core/sammyseq pipeline implemented in Nextflow to generate genome-wide signal tracks and paired comparisons. We analyzed a contact-based matrix derived from individual fractions to define nucleoid domains.

We adapted 4f-SAMMY-seq to *Helicobacter pylori*, confirming its ability to capture DNA domains according to their different accessibility also in bacteria. We achieved a kilobase domain resolution with 500000 reads per fraction and observed specific signals for some promoters opening the possibility to further decrease domains resolution.

4f-SAMMY-seq promises an advantageous alternative for studying nucleoid accessibility, and to further assess its potential, we will integrate transcriptomic and ChIP-seq data.

A32

Role of the diadenosine tetraphosphate hydrolase ApaH in the control of *Pseudomonas aeruginosa* virulence

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The intracellular signaling molecule diadenosine tetraphosphate (Ap4A) has been implicated in regulating stress responses, antibiotic resistance, and virulence-associated traits in several bacterial species. However, its role in the human pathogen *Pseudomonas aeruginosa* has remained unclear. By combining genetic, biochemical, transcriptomic, and phenotypic analyses, we demonstrated that a *P. aeruginosa* mutant lacking the Ap4A-hydrolyzing enzyme ApaH accumulates high intracellular levels of Ap4A and is drastically impaired in the production of multiple virulence factors and in pathogenicity in different infection models. Deletion of *apaH* in *P. aeruginosa* clinical isolates confirmed that the positive effect of ApaH on virulence gene expression and infectivity is broadly conserved, highlighting the potential of *P. aeruginosa* ApaH as a target for antivirulence therapies. Biochemical and structural studies identified potential key residues responsible for ApaH catalytic activity, and *in vivo* expression assays confirmed their crucial role in ApaH capability to control *P. aeruginosa* virulence. In the model organism *Escherichia coli*, Ap4A can bind to specific proteins, such as chaperones, stress-response regulators, and metabolic enzymes, and can also be incorporated by the RNA polymerase into nascent transcripts as a protective 5'-cap, which can be removed by ApaH and the RNA pyrophosphohydrolase RppH. Knockout and cross-complementation experiments revealed that the effect of ApaH on *P. aeruginosa* virulence is only partially influenced by the deletion or overexpression of the *P. aeruginosa* RppH homolog, suggesting that ApaH may influence virulence through both RNA capping-dependent and -independent mechanisms in *P. aeruginosa*.

A33

VOMG, a new drug candidate against *Mycobacterium abscessus*, *Staphylococcus aureus* and other microbial pathogens

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Among the fast-growing non-tuberculosis mycobacteria (NTM), *Mycobacterium abscessus* (*Mab*) poses a major threat to immunocompromised individuals, including those with cystic fibrosis, due to limited antibiotic options. VOMG is a new small synthetic, water-soluble compound showing high bactericidal activity against *Mab* growth (MIC=0.25 µg/ml) and is also active against *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus* and fungi.

Transcriptomic analysis revealed downregulation of genes encoding cell division (CD) proteins such as FtsZ, SepF, EnvC, SteA, FtsQ. Using the CRISPRi system with integrative vectors (pLJR962 and pLJR965 modified with zeocin cassette), we generated conditional knock-down (KD) mutants. In these mutants, the genes expression is modulated by the addition of anhydrotetracycline (ATc). ATc titration confirmed that *ftsZ* is essential for *in vitro* growth. Upon *ftsZ* depletion in liquid medium CFU/mL decreased significantly in the conditional mutants in the presence of ATc, demonstrating that *ftsZ* depletion is bactericidal. We tested VOMG against *Mab ftsZ* KD mutants by resazurin microtiter assay; these mutants are more sensitive to VOMG than their parental strains (2X MIC), confirming FtsZ as VOMG target. The other KD mutants in CD genes are under development.

Interestingly, encouraging preliminary studies also showed promising efficacy of VOMG in mouse model of *S. aureus* sepsis, highlighting the potential of VOMG as a promising therapeutic agent. Finally, its properties suggest potential application in treating urinary tract infections.

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A34

How genome mining can guide the discovery of novel glycopeptide antibiotics: kineomicins model

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Antimicrobial resistance (AMR) is one of the most urgent global health threats. Each year, infections caused by multi-drug resistant (MDR) bacteria are responsible for approximately 1.3 million deaths, a number projected to rise to 10 million annually by 2050 without effective actions, calling for the discovery of new antibiotics. To expand the current arsenal of glycopeptide antibiotics (GPAs) -clinically used to treat infections caused by MDR Gram-positive pathogens- genome mining has emerged as a powerful strategy for identifying novel chemical entities and their newly producer strains^[1]. In this context, we explored 600 genomes of the order *Pseudonocardiales* and identified two biosynthetic gene clusters (BGCs) encoding for putatively unknown GPAs^[2]. The first antibiotic, named kineomicins from its producer strain *Actinokineospora auranticolor*, was produced up to an exceptionally high production rate (>1 g/L in a benchtop bioreactor). The antibiotic complex was microbiologically characterized and the structure of its main congener, KmcB, resolved by LC-MS, MS/MS, and NMR spectroscopy, confirming that the molecule has a unique peptide scaffold^[3]. More recently, we started working with *Umezawaea endophytica*, the producer of the second identified putative GPA, which is being cultivated in various media to assess its antibiotic production potential. The aim is to further validate the genome mining approach as a key strategy in the discovery and development of new antibiotics to tackle AMR spread.

^[1]Yushchuk et al. 2021. *ACS Chem Biol.* 16(5):915-928

^[2]Andreo-Vidal et al. 2021. *Antibiotics (Basel).* 10(12):1533

^[3]Yushchuk et al. 2025. *Commun Chem.* 8(1):134

A35

Investigating de novo L-cysteine biosynthesis in *Pseudomonas aeruginosa* for the development of new antimicrobials

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Differently from humans, most bacteria assimilate inorganic sulfur through the reductive sulfate assimilation pathway or *de novo* L-cysteine biosynthesis (DeNoCB). This peculiarity makes DeNoCB an appealing target for antimicrobial development, with promising results already observed with several bacterial species. Surprisingly, in *Pseudomonas aeruginosa* this pathway has been overlooked and remains completely unexplored for antibiotic discovery.

This study focuses on the identification and physiological/biochemical characterization of key DeNoCB enzymes. Through the generation of *P. aeruginosa* deletion mutant(s) the role of CysK (PA2709), CysM (PA0932), CysE (PA3816) and CysH (PA1756) has been investigated.

The identified CysK and CysM represent the two main isoforms of cysteine synthase catalyzing the final step of DeNoCB, allowing bacteria to proliferate in minimal media supplemented with either sulfate or thiosulfate. Single and double deletion mutants ($\Delta cysM$, $\Delta cysK$ and $\Delta cysM\Delta cysK$) were generated to highlight *P. aeruginosa* substrate preference. Conversely from the $\Delta cysM\Delta cysK$ mutant, deletion of either one of the two genes did not lead to cysteine auxotrophy. However, in the presence of thiosulfate as the only sulfur source, $\Delta cysM$ behaved as a cysteine bradytroph, indicating that another pathway is likely activated. Moreover, the key role of CysE in supplying O-acetylserine has been highlighted, as the $\Delta cysE$ mutant is unable to grow with either sulfate or thiosulfate as sole S-source. Finally, the cysteine auxotrophy of the $\Delta cysH$ mutant has also been confirmed. The parallel recombinant production and functional characterization of the purified enzymes is allowing the selection of inhibitors through compound library screening, whose validation is currently ongoing.

A36 DpaA and Lpp affect LD-cross-link formation in *Escherichia coli*

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Peptidoglycan (PG) is an essential component of the cell envelope, required to mechanically sustain the turgor pressure of the cytoplasm. In *Escherichia coli*, the PG consists of glycan chains with attached stem peptides mainly cross-linked via DD-cross-links formed between Alanine (D-Ala⁴) and meso-diaminopimelic acid (DAP³) by DD transpeptidases. A small amount of LD-cross-links occurs between two DAP³ residues, formed by LD-transpeptidases members of the YkuD family of proteins. Although not essential under standard laboratory conditions, LD-cross-links become crucial for β -lactam resistance, PG homeostasis and survival under stress conditions. *E. coli* has two LD transpeptidases, LdtD and LdtE for LD-cross-link formation. LdtD is the cell envelope-stress-induced LDT and LdtE is the housekeeping LDT active under non-stress conditions. Interestingly, the formation of LD-cross-links by LdtE requires DpaA, another YkuD protein family member, which detaches the outer membrane (OM)-anchored lipoprotein Lpp from the PG. Lpp (Braun lipoprotein) is the most abundant OM lipoprotein which is attached to PG via its terminal Lys and a DAP³ residue in the PG. This is the only covalent linkage between the PG and OM and is needed to maintain a robust cell envelope. By analysing the PG composition of wild type and mutants lacking *ldt* genes, *dpaA*, and/or *lpp*, we found that DpaA and Lpp are both required for LD-cross-link formation by LdtE, highlighting the unexpected functional interaction between Lpp detachment dynamics and LD-cross-link formation.

A37

Characterization of the de novo cysteine biosynthetic pathway in *Pseudomonas aeruginosa*M. Mellini¹, M. Beccarini¹, C. Ridolfi¹, L. Leoni¹, G. Rampioni^{1,2}¹Department of Science, University Roma Tre, Italy²IRCCS Fondazione Santa Lucia, Rome, Italy

The increasing incidence of infections caused by multidrug-resistant bacteria underscores the urgent need for alternative therapeutic strategies. In this context, the identification of novel drug targets is a key research priority. Recent studies have highlighted the *de novo* cysteine biosynthesis pathway as a crucial contributor to bacterial processes such as virulence and antibiotic tolerance in various species. Consequently, this pathway is emerging as a promising target for the development of antivirulence agents and antibiotic adjuvants.

In *Pseudomonas aeruginosa*, a major opportunistic pathogen, the *de novo* cysteine biosynthetic pathway remains poorly characterized, despite the presence of genes predicted to encode the associated enzymes.

This study aimed to functionally characterize this pathway in *P. aeruginosa* and assess its role in pathogenicity. Mutants lacking genes putatively involved in cysteine biosynthesis were generated and evaluated for: *i*) growth in different media with or without cysteine supplementation; *ii*) production of major virulence factors *in vitro*; *iii*) pathogenicity in both plant and animal infection models.

Genes essential for growth in cysteine-depleted conditions have been identified. Interestingly, some mutants also exhibited growth defects in standard cysteine-rich media and in media mimicking the host environment. Notably, specific mutations led to reduced virulence *in vitro* and *in vivo*, despite unaltered growth, suggesting a direct role in pathogenesis beyond cysteine supply.

These findings highlight the functional relevance of the *de novo* cysteine biosynthesis pathway in *P. aeruginosa* patho-physiology, underscoring its potential as a therapeutic target.

A38

Genome-Wide Association Analysis Reveals Niche-Specific Genes in *Escherichia coli*R. Nodari^{1,2}, L. Sterzi³, D.M. Minore³, C. Bonaiti³, G. Bettoni³, L. Folgori⁴, L. Barcellini⁴, S. Panelli³, R. De Francesco^{1,2}, F. Comandatore³¹Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Italy²INGM - Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy³Department of Biomedical and Clinical Sciences, Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Italy⁴Department of Paediatrics, Vittore Buzzi Children's Hospital, Milan, Italy

Escherichia coli is a Gram-negative bacterium commonly found in the human gut as a harmless commensal, where it aids nutrient absorption and gut homeostasis. However, under certain conditions, it can act as an opportunistic pathogen, particularly when it migrates to sterile body sites such as the urinary tract or bloodstream, causing urinary tract infections (UTIs), bloodstream infections (BSIs), or sepsis. Neonates are especially vulnerable to *E. coli*-associated sepsis due to their immature immune systems. This shift in pathogenic potential is largely driven by the accessory genome, which encodes virulence and resistance factors that promote survival in diverse environments.

In this study, we performed a genome-wide analysis of 587 *E. coli* genomes isolated from diverse sources, including blood, urine, hospital environments, and natural settings. After phylogenetic clustering, we applied a gene presence/absence GWAS to identify genetic traits associated with isolation source, independent of lineage. Among the ~200 annotated protein-coding genes positively associated with one or more sources, virulence genes involved in adhesion, ion transport, and immune evasion were, as expected, predominantly linked to blood and urine isolates, with some differences between adults and neonates. Interestingly, genomes from hospital environments showed a marked enrichment in antibiotic resistance genes, underscoring the strong selective pressure imposed by healthcare settings and the use of antibiotics. These findings highlight source-specific genetic adaptations in *E. coli*, offering insights into molecular mechanisms underlying colonization and infection. The identified genes may serve as valuable markers for predicting pathogenic potential, particularly in vulnerable hosts such as neonates.

A39 Response of *Escherichia coli* to blue light stress

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Antimicrobial Blue Light (aBL) is a technique that can be used to control the growth of pathogens and contaminants in several applicative fields, from sanitization of inert surfaces to human skin treatments, from industry to food and feed. Even if the mechanism of action has not yet been elucidated, it has been hypothesized that specific wavelengths can activate potential endogenous photosensitizers in microbial cytoplasm and/or envelope. The arisen photooxidative stress could compromise macromolecules and induce inactivation of microorganisms.

In this scenario, we compared the response to light at 410 nm of *Escherichia coli* K-12 MG1655 and JM109 strains. The wild type MG1655 showed to be significantly more tolerant to blue light irradiation than the isogenic derivative JM109 strain. It is well-known that recombinase A (*recA*) is directly involved in the repair of DNA caused by reactive oxygen species (ROS). Since *E. coli* JM109 carries a *recA1* point mutation which renders the Recombinase A non-functional, we evaluated the effect of the recombinant expression of the wild type RecA on blue light sensitivity. Indeed, JM109 reverted to a higher tolerance comparable to that of MG1655. In addition, to investigate which ROS could be involved in the aBL mechanism, we overexpressed two genetic determinants from *Pseudomonas aeruginosa* PAO1 codifying Catalase A (*KatA*) and Superoxide dismutase B (*SodB*), respectively. Upon blue light irradiation, *E. coli* JM109 was protected by both enzymes supporting the potential involvement of hydrogen peroxide and superoxide anion. These results emphasize the importance of further research in aBL mechanism.

A40

Optimization of EUCAST MIC Testing for *Mycobacterium tuberculosis*: The Role of Inoculum Size

On behalf of ERA4TB – EUCAST multicentre study group:

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ERA4TB is a multi-institutional, multi-disciplinary platform supporting anti-tuberculosis drug development from preclinical to phase 1 trials, producing regulatory-grade data. Seven ERA4TB institutions implemented a standardized EUCAST-based platform to determine MICs, ECOFFs, and clinical breakpoints, following EUCAST SOPs.

Technical concerns have arisen with EUCAST SOPs, particularly regarding inoculum size. SOP version 6.1, active since 2019, required 5×10^4 to 5×10^5 CFU/mL, with deviations invalidating MIC results. Despite harmonized protocols and materials, our platform initially showed variability in inoculum levels. Adjustments—such as controlling agar plate moisture, inoculum spreading, and vortexing—improved consistency across labs.

Considering our findings, EUCAST later revised its SOPs (versions 7.0, 8.1, and 8.2). Our consortium contributed to the public consultation for version 7.0. In version 8.1, the inoculum range was broadened to 1×10^4 – 1×10^6 CFU/mL based on practical experience, but without supporting data. We evaluated how this change impacted MIC determinations for isoniazid (v8.1) and later for isoniazid and diarylquinolines (v8.2, released January 2025).

Our data showed that inoculum size affected MIC interpretation under version 8.1; however, version 8.2 resolved this variability. Preliminary ERA4TB data confirmed stable MIC values across the broader inoculum range.

Version 8.2 also introduced improved guidance on plate readouts, incubation times, visual interpretation, and standardized procedures for media and drug preparation, contributing to more reproducible and reliable MIC results across laboratories.

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853989.

A41	Development of abietane-based ArnT inhibitors to block colistin resistance <i>Pseudomonas aeruginosa</i>
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Colistin is a last-resort antibiotic against multidrug-resistant Gram-negative bacteria. However, resistance to colistin is spreading and increases the risk that this antibiotic will rapidly become ineffective. Colistin resistance rely on modification of the lipid A component of the outer membrane, which is the target of colistin. In *Pseudomonas aeruginosa* this is mainly determined by the *arn* operon, whose last enzyme, the transferase ArnT, mediates the addition of 4-Amino-4-deoxy-l-arabinose to the lipid A.

Based on our previous identification of the natural *ent*-beyerene diterpene FDO as an ArnT inhibitor, (doi:10.1021/acs.joc.0c01459; 10.1093/jac/dkaa200), a rational design was applied to simplify the *ent*-beyerene scaffold up to drug-like synthetic ArnT inhibitors. Starting from the aromatic abietane scaffold of the podocarpic acid, twenty-four semisynthetic oxygen and nitrogen abietane-based derivatives were prepared. Four of them (2, 13, 18, and 30) significantly restored colistin activity against colistin-resistant *P. aeruginosa* strains, with compound 18 showing the lowest IC₉₀ (182.5 μM). To rule out a possible effect of the compounds on membrane permeability, which in turn may potentiate colistin activity, the uptake of 1-N-phenyl-naphthylamine (NPN), which is indicative of bacterial outer membrane permeabilization, was evaluated. As expected, colistin increased NPN uptake in colistin-susceptible, but not in resistant strains. In contrast, compounds 2, 13, 18, and 30 did not induce NPN uptake, nor did they alter colistin-induced uptake when co-administered with colistin. These results are consistent with the ArnT inhibitory activity of abietic acid-derived compounds, highlighting the key role of the abietan aromatic skeleton in restoring colistin sensitivity in *P. aeruginosa*.

A42	Role of the <i>yajC-secDF</i> operon in <i>Burkholderia</i>
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SecDF proteins, included in the RND family of efflux pumps, are present both in Bacteria and Archaea. Some roles have been suggested for this system in different microorganisms, from protein translocation across or into the inner membrane to antibiotic resistance. However, its function in bacterial cells remains elusive. Although this system proved to be non-essential for cells viability in most bacteria, recently, the use of TnSeq revealed that the genes encoding SecDF are essential in various bacteria, including *Burkholderia* spp.

Since essential genes are considered excellent targets for new antimicrobial molecules, our aim is to understand the role of the SecDF system in *Burkholderia* species to evaluate its possible use as new antibiotics target.

The two proteins are encoded by an operon that also contains the *yajC* gene. A *yajC-secD-secF* rhamnose-inducible conditional mutant of *Burkholderia cenocepacia* K56-2 has been constructed, allowing us to confirm the essentiality of this operon in this strain. This mutant has been used also to assess some phenotypes associated with SecDF in other microorganisms and to perform transcriptomic experiments in presence of different rhamnose concentrations.

Preliminary results indicate that the absence of this system determines:

- A down-regulation of protein synthesis and of flagellar assembly and chemotaxis.
- An up-regulation of alternative sigma factors associated with stress (RpoE and RpoH) and of some amino acid degradation pathways.

All the data that we are collecting will allow us to formulate some hypotheses, which will then be validated, on the possible role of this system in *Burkholderia*.

A43

Snapshots of nucleoid dynamics in *Helicobacter pylori* thanks to 4f-SAMMY-seq, a hi-C alternative to map chromosome accessibility

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Four fraction Sequential Analysis of MacroMolecules Accessibility (4f-SAMMY-seq) is a new genome-wide sequencing-based technique to map chromosome accessibility that, in eukaria, proved to be powerful as HiC without requiring restriction enzymes or crosslinking. Its application to bacteria can improve cross-species comparisons and foster the study of chromosome conformation toward the still unexplored field of nucleoid dynamics.

This study aims to adopt 4f-SAMMY-seq to investigate chromosome conformation dynamics in *H. pylori* comparing different growth phases. We will then investigate the correlation between these dynamics, gene expression regulation and functional alterations integrating them with multiple -omic data.

4f-SAMMY-seq relies on the biochemical separation of DNA into sequential fractions with decreasing accessibility. Fractions are sequenced and a contact-based matrix can be derived to define chromosome domains using standard Hi-C analysis tools, while DNA accessibility alterations across conditions can be measured comparing the relative ratios of fraction pairs.

We obtained a fine map of bacterial chromosome accessibility with just 2000000 reads per sample. We defined the dynamic alterations of nucleoid structure among different growth conditions, revealing both accessible and not-accessible DNA signals alterations, and we are correlating these data to gene expression and/or transcription factor binding site annotations to further interpret our results.

We applied for the first time to bacteria 4f-SAMMY-seq technique, which is now available to investigate different prokaryotes species, and proved its ability to provide high-resolution chromosome accessibility maps to capture nucleoid dynamics avoiding major Hi-C bias.

A44 Genetic epidemiology of neomycin-resistant *Escherichia coli* across European livestock

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Background: Neomycin, a broad-spectrum aminoglycoside, is widely used in livestock to control colibacillosis. Resistance among *Escherichia coli* isolates is increasing, likely driven by the expanded use of neomycin following the European restrictions on zinc oxide and colistin.

Objectives: To investigate the genetic epidemiology of neomycin-resistant *E. coli* across diverse livestock sources and five European countries.

Methods: A total of 750 recent neomycin-resistant *E. coli* isolates were included through retrospective genome screening and prospective isolation from livestock. Genomes analysis on both short- (70.5%) and long-reads (29.5%) data included phylogenetic comparison, identification of neomycin resistance genes and analysis of their genetic contexts.

Results: neomycin resistance was mediated by *aph(3')-Ia* in 98.2% of isolates, which were distributed across 28 clonal complexes (CC), predominantly CC10, which was highly frequent across all countries (19.3-30.2%) and hosts (11.1-27.5%). Genetic context analysis revealed that *aph(3')-Ia* was primarily carried by conjugative or mobilizable plasmids (70.3%) within five distinct genetic contexts, including the known transposons Tn903 and Tn4352. A chromosomal integrated variant accounted for the remaining cases (29.7%). Plasmids carrying *aph(3')-Ia* frequently co-harbored genes conferring resistance to tetracyclines and trimethoprim-sulfamethoxazole, with IncX1 plasmids originating in *Salmonella enterica* which additionally carried chloramphenicol and quinolones resistance determinants. Phylogenetic analysis revealed broad dissemination of plasmid-associated variants across multiple lineages, while the chromosomal variant was mostly restricted to specific lineages (ST117, ST88, ST189), indicating vertical transmission.

Conclusions: Conjugative plasmids play a key role in the spread of neomycin resistance among European livestock, facilitating the co-transmission of other clinically important antimicrobial resistance determinants.

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A45	The Lpp detaching enzyme DpaA post-translationally controls the level of PG amidase activator ActS to enable survival under severe OM biogenesis defect in <i>Escherichia coli</i>
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The coordinated growth of the three-layered cell envelope of Gram-negative bacteria is critical under stress conditions or when the assembly of one of the layers is compromised. In *Escherichia coli*, when the export of lipopolysaccharide (LPS) to the outer membrane (OM) is defective, the DpaA protein is required to avoid cell lysis. DpaA is an enzyme that detaches the lipoprotein Lpp from peptidoglycan (PG), a non-essential function under normal growth conditions. The lysis of LPS export stressed cells lacking DpaA does not happen in the absence of ActS, a LytM protein that contributes to the activation of PG amidases, which hydrolyze septal PG to enable separation of daughter cells. As ActS is dispensable under non-stress conditions it is believed that in OM stressed cells lacking DpaA, spurious activation of amidases through ActS contribute to cell lysis. However, how the functional interaction between DpaA and ActS prevents cell lysis under envelope stress is not understood. Our recent data suggest that the Lpp detachment activity of DpaA is not required to allow cell survival under stress and that spurious activation of amidases does not depend on increased expression of *actS* under stress conditions. Instead, we found that under stress conditions and in a DpaA dependent way, the cell keeps the level of ActS low implicating a novel post-translational mechanism to control the relative abundance of ActS in the cell.

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A46	Investigating Bedaquiline Mechanisms of Action and Resistance: A Focus on MmpL4/MmpL5 Siderophore Transporters and the Novel Diarylquinoline TBAJ-587
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TBAJ-587, a preclinical derivative of bedaquiline (BDQ) developed by TB Alliance, demonstrates improved safety and efficacy profiles. Like BDQ, it targets the ATP synthase F₀F₁ complex by inhibiting the *atpE*-encoded subunit and shares the same resistance mechanism via MmpL5 efflux pump overexpression caused by *Rv0678* mutations. In preclinical models, TBAJ-587 is metabolised into three active metabolites (M2, M12, M3), with M3 being the most abundant. Starting from an MDR *Rv0678* mutant, we isolated two *M. tuberculosis* mutants harboring a deletion in the *mmpL4* gene (Δ G1921), resistant to the 3 metabolites but sensitive to BDQ and TBAJ-587. *mmpL4* is not essential for *in vitro* growth but is required for *in vivo* virulence.

Both MmpL4 and MmpL5 are important for siderophore transport; if one is absent, the other complements its role. Furthermore, MmpL5 plays the main role in BDQ efflux.

We are investigating whether MmpL4 functions as an M3 efflux pump or secondary target.

In untreated *Rv0678* mutant, RT-qPCR revealed only the overexpression of *mmpL5*. Upon M3 exposure, both *mmpL4* and *mmpL5* were upregulated, suggesting that M3 may induce *mmpL4* expression. Chromium Azurol S (CAS) assays showed that M3 does not impair MmpL4's siderophore export, because of MmpL5 presence.

Ongoing RNA-seq aim to elucidate the precise role of MmpL4 in mediating resistance to M3. These findings could help for next-generation TB therapies by identifying novel resistance mechanisms and potential drug targets.

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853989.

A47

CUT&Tag in *Mycobacterium tuberculosis* Reveals Unconventional Genomic Landscape in Response to Oxidative Stress

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, remains a global health threat due to increasing drug resistance and high mortality rates. This study introduces the first application of Cleavage Under Targets and Tagmentation (CUT&Tag)¹ in bacteria, focusing on mapping G-quadruplexes (G4s) in *Mtb* under standard and oxidative stress conditions. CUT&Tag, an advanced genomic profiling technique, offers superior sensitivity, specificity, and reproducibility compared to traditional methods like ChIP-seq, while requiring less input material and sequencing depth. Here, we validated the CUT&Tag protocol using an antibody against the RNA polymerase β -subunit, confirming its association with actively transcribed genes and demonstrating enhanced efficacy compared to previous ChIP-seq analyses².

Employing the anti-G4 antibody BG4³, we revealed that *Mtb* G4s, unlike their eukaryotic counterparts, predominantly localize within gene coding sequences and consist of two-guanine tract motifs. Importantly, oxidative stress conditions, which mimic the intracellular environment *Mtb* encounters in macrophages, induced increased G4 formation. This correlated with a transcriptional block at G4 sites, suggesting a potential role for G4s in bacterial stress response and survival within host cells.

Our findings provide the first in vivo evidence of G4 formation in the *Mtb* genome and unveil an unconventional G4 landscape. This study not only demonstrates the successful application of CUT&Tag in bacteria but also offers new insights into bacterial stress response mechanisms, potentially identifying novel therapeutic targets for combating tuberculosis.

References

¹Zanin, I. et al. Genome-wide mapping of i-motifs reveals their association with transcription regulation in live human cells. *Nucleic Acids Res.* 51, 8309–8321 (2023).

²Uplekar, S., Rougemont, J., Cole, S. T. & Sala, C. High-resolution transcriptome and genome-wide dynamics of RNA polymerase and NusA in *Mycobacterium tuberculosis*. *Nucleic Acids Res.* 41, 961–977 (2013).

³Maurizio, I. et al. Production of the anti-G-quadruplex antibody BG4 for efficient genome-wide analyses: From plasmid quality control to antibody validation. *Methods Enzymol.* 695, 193–219 (2024).

A48

Involvement of nanotubes in *Bacillus subtilis* and *Staphylococcus aureus* competitionA. Saggese¹, Y. De Luca¹, E. Ricca¹, L. Baccigalupi²¹Department of Biology, Federico II University, Naples, Italy²Department of Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy

Bacteria have developed different mechanisms to compete with other species based on either the secretion of antimicrobials in the extracellular environment or the direct transfer of toxic compounds from a donor to a target cytoplasm. In this context, the gram-positive bacterium *Bacillus subtilis* efficiently fight competitors by using both systems. It secretes a variety of antimicrobials active against many target microorganisms^[1] and directly delivers toxins to the competitor cytoplasm through the Type 7 secretion System and nanotubes^[2]. Indeed, *B. subtilis* cells have been shown able to successfully compete with *B. megaterium* through the nanotube-based delivery of the toxin WapA and reduce *Staphylococcus aureus* colonization *in vivo* inhibiting the pathogen's quorum-sensing signalling^[3,4]. Here, we report that *B. subtilis* is also able to inhibit the growth of several strains of *S. aureus*, including multi-drug resistant ones (MRSA) through a contact-dependent mechanism. Such anti-*S. aureus* activity relies on the biosynthesis of nanotubes but the transferred molecule is not the anti-*B. megaterium* toxin WapA. The analysis several *B. subtilis* strains showed that the Natto strain was the only one unable to compete with *S. aureus*. A comparative genomic analysis is in progress to identify potential candidate/s responsible of the toxic activity.

^[1]Antibiotics (Basel). 2022, 12;11(1):88 • ^[2]mBio. 2022, 26;13(5):e0013422 • ^[3]Nat Commun. 2017, 22;8(1):315

^[4]mSystems. 2024, 20;9(8):e0071224

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A49

Identification of bacteriocin-mediated antimicrobial activity of *Streptococcus salivarius* 24SMBc against different pneumococcal serotypes

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The probiotic strain *Streptococcus salivarius* 24SMBc is characterized by strong antimicrobial activity against pathogenic *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In this work we evaluated the effects of disrupting bacteriocin genes *blpU* and *blpK* on the antimicrobial activity of *S. salivarius* 24SMBc. Then, we tested *BlpU* against different serotypes of *S. pneumoniae*. Knockout mutants of bacteriocin genes ($\Delta blpU::Spec^R$, $\Delta blpK::cat$, $\Delta blpU::Spec^R-\Delta blpK::cat$) were constructed by natural transformation. Antimicrobial activity of *S. salivarius* 24SMBc and its derivative strains was tested against *Streptococcus* spp. by spot-on-lawn assay. *BlpU* was isolated with acetone precipitation of 24SMBc cell-free supernatant (CFS) and quantified by a modified spot-on-lawn assay, comparing the inhibition halo of CFS proteins with a synthetic form of *BlpU* at scalar dilutions. Similarly, MIC and MBC of synthetic *BlpU* was measured for different *S. pneumoniae* serotypes (NT, 9V, 15C, 19A, 19F).

Spot-on-lawn assays showed that *BlpU* is the bacteriocin involved in *S. salivarius* 24SMBc antimicrobial activity, as the strain $\Delta blpU::Spec^R$ and the double mutant $\Delta blpU::Spec^R-\Delta blpK::cat$ show a complete loss of activity. *BlpU* has a broad spectrum of activity against several streptococci including *S. pneumoniae*. Active *BlpU* was recovered from the CFS, and its production reached

a concentration of 38 mM at 24 hrs. Furthermore, MIC values for BlpU (3.12-1.56 μM) and MBC (6.25-3.12 μM) confirmed the strong bactericidal activity against different *S. pneumoniae* serotypes at low concentrations.

In conclusion, we identified BlpU as the main player in bacteriocin-mediated antimicrobial activity of *S. salivarius* 24SMBc and suggest its potential applications against *S. pneumoniae*.

A50	More than membrane building blocks: how Fatty Acid Degradation fuels Bacterial Adaptation
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The environmental conditions and nutrient availability dictate the metabolic state of bacterial cells. In the absence of growth-supporting substrates or under stress conditions, bacteria enter stationary phase, during which they must exploit alternative carbon sources. Previous studies have suggested that bacteria undergo an autophagy-like process, where endogenous macromolecules may serve as carbon substrates for energy production. During stationary phase, fatty acid degradation (*fad*) genes are upregulated, suggesting that fatty acids may serve as potential energy sources. Through lipid extraction and analysis, we demonstrated that, during stationary phase, endogenous fatty acids are released from membrane lipids through the activity of two lipases: the L2-lysophospholipase PldB and the patatin-like lipase YjjU. Once in the cell, these fatty acids can serve as substrates for the Fad machinery, contributing to bacterial survival under nutrient-deprived conditions. Notably, *fad* mutants exhibit reduced viability during stationary phase, further supporting the role of an autophagy-like process. Additionally, during stationary phase, *E. coli* undergoes membrane lipid remodeling, which correlates with an enhanced resistance to certain membrane-targeting antibiotics, such as vancomycin. However, neither the *yjjU* mutant nor the *fad* mutants exhibited increased resistance to vancomycin over time. These findings suggest that fatty acid metabolism is not only closely linked to energy production but also to membrane remodeling, highlighting a potential connection between lipid homeostasis and bacterial adaptation to nutrient scarcity.

A51	A virtual screen-based approach to identify bacterial cell division inhibitors against ESKAPE infections
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Multi-drug resistant (MDR) ESKAPE pathogens are well-known in nosocomial and community-associated infections, characterised by high levels of morbidity and mortality. In the search for new compounds able to tackle MDR strains, FtsZ inhibitors represent a valuable option, so the divisome proteins FtsZ and FtsA/FtsN were targeted applying Computer Aided Drug Design (CADD) methods.

Libraries of natural and non-natural compounds were docked to the cofactor binding sites: twenty-four molecules were obtained from the virtual screening on FtsZ, among which C11 was demonstrated to alter FtsZ polymerization in vitro. C11 is a bacteriostatic molecule with an MIC of 2 $\mu\text{g}/\text{ml}$ against *S. aureus* and MRSA clinical isolates. Single-cell time-lapse microscopy

performed in the presence of C11 showed FtsZ delocalization. The combination of C11 with β -lactams restores methicillin susceptibility to MRSA strains. Confocal laser scanning microscopy showed that C11 can inhibit and eradicate *S. aureus* biofilms. Moreover, C11 increases survival in the *Galleria mellonella* infection model, and shortly it will be tested in mice.

On the other hand, 20 compounds resulted from the virtual screening on FtsA/FtsN interaction. Their inhibition against FtsA ATPase activity and their ability to interfere with the FtsA/FtsN interaction were evaluated using spectrophotometric and pull-down assays. None of the compounds blocks FtsA ATPase activity, but four of them interfere with FtsA/FtsN. Further investigations will be necessary to improve their efficacy.

Taken together, our results indicate that virtual screening is an interesting and fast method to identify new antibacterial molecules.

A52

Multidrug-resistant *Klebsiella pneumoniae*: plasmid mediated carbapenemes' gene transfer

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Klebsiella pneumoniae is the second leading cause of Gram-negative bacteraemia, and the third leading cause of bloodstream infection (BSI) overall. Most of BSIs in hospital settings are caused by multidrug-resistant (MDR) *K. pneumoniae* strains that are also resistant to carbapenems and third-generation cephalosporins, which are considered last-resort antibiotics. Resistance to carbapenems is often associated with the acquisition of genes that code for imipenemase (IMP), the Oxacillinase-48 (OXA-48) type carbapenemases, the New Delhi Metallo-beta-lactamase (NDM), and the *K. pneumoniae* carbapenemase (KPC). These genes are mainly located on plasmids which can mediate the horizontal transfer of carbapenemase-coding genes.

The data presented here are part of the PRIN2022PNRR project entitled “A snapshot of transferable plasmids based on omics and clonal epidemiology in hospital-acquired carbapenem-resistant *Enterobacterales*: a pilot study” (Funded by European Union-Next Generation EU, Mission 4 Component 2 CUP H53D23007510001 Cod P2022RHYTM) and are focused on genomic features of 20 MDR *K. pneumoniae* strains causing BSI. The strains were collected in 2024 by the surveillance observatory laboratories present in the polyclinic hospitals of Bari and Catania (Italy). Characterisation for carbapenemase-coding genes revealed the presence of the bla_{KPC-3} on IncFIB(pQil)/IncFII(K) plasmids; bla_{OXA-48} on IncL plasmids; and bla_{NDM-1} and bla_{NDM-3} on IncFIB(pQil). Inter-species horizontal gene transfer using *Escherichia coli* as recipient showed low frequency of transfer (1×10^{-5} – 1×10^{-6} transconjugants/recipient) for bla_{KPC-3}, high frequency (1×10^{-1} – 1×10^{-2} transconjugants/recipient) for bla_{OXA-48} and no detectable transfer ($\leq 1 \times 10^{-2}$ transconjugants/recipient) for bla_{NDM-1} and bla_{NDM-3}. Results are part of a larger study which will imply integration of plasmidome data in phylogenomics analyses.

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A53	Comparative genomics analyses of <i>Bifidobacterium adolescentis</i> revealed links between the genetic variability of this human gut microbial species with geographic origin
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Among the genus *Bifidobacterium*, the *Bifidobacterium adolescentis* species is one of the most frequently associated taxon to the human gut microbiota of adults, second only to the *Bifidobacterium longum* species. Unlike the latter taxon, *B. adolescentis* has not been extensively studied and characterized, leaving gaps in our understanding of its physiological traits, genetic diversity, and potential interactions within the other members of the human gut microbiota. In this study, a dataset of 1682 *B. adolescentis* genomes was compiled by combining data from public available repositories and metagenome assemblies of 131 projects to unveil the unique characteristics of this species in comparison with other *Bifidobacterium* species. A pangenome analysis of *B. adolescentis* has allowed the identification of 203 Clusters of Orthologous Genes (COGs) absent in any other bifidobacterial species colonizing humans, six of which characterized by unique functional annotations. Furthermore, several COGs were found to have undergone gene acquisition events, encoding extracellular structures involved in the interaction with the host and other microorganisms, such as pili, as well as defence mechanisms against phage infections represented by CRISPR systems. Additional phylogenetic analyses revealed seven sequence-based clusters within the *B. adolescentis* species, each partially associated with the ecological origin of the strains, suggesting phylogenetic differentiation potentially shaped by the geographical origin of the analyzed genomes.

A54	Exploring the envelope stress responses of <i>Escherichia coli</i> upon treatment with the Lpt-targeting antimicrobial peptide thanatin
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Gram-negative bacteria (GNB) are a major cause of antibiotic-resistant infections due to their highly impermeable cell envelope containing lipopolysaccharide (LPS), which prevents antibiotics from entering the cell.

The biogenesis of LPS relies on the activity of the essential LPS transport machinery (Lpt), which is an attractive target for antibiotics.

The antimicrobial peptide thanatin targets the Lpt machinery, resulting in membrane defects and cell death. However, thanatin is not a suitable drug candidate due to its poor drug-like properties and the rapid emergence of resistance. Therefore, understanding the mechanisms of resistance to thanatin is crucial for developing novel antimicrobial strategies against GNB.

This study aimed to explore the mechanism of bacterial adaptation to thanatin through transcriptomics and physiological analyses.

The study revealed differential expression of the two-component systems CpxAR and RcsCD following thanatin treatment.

In contrast to polymyxin, a potent inducer of Rcs, treatment with thanatin caused rapid activation of the CpxAR system, followed by the RcsCD and FlhDC systems. Functional characterisation of CpxAR-regulated genes revealed an enrichment of pathways involved in cationic peptide resistance, stress regulation, protein folding, and peptidoglycan remodelling. Within the RcsCD regulon, genes involved in capsule synthesis were found to be upregulated. Conversely, FlhDC-controlled motility genes were downregulated. This shift towards reduced flagellin expression alongside increased capsule biosynthesis suggests a potential transition towards a sessile lifestyle, possibly as a resistance strategy.

This study sheds light on the specific stress response pathways activated by thanatin exposure, thereby advancing our understanding of the mechanisms underlying resistance acquisition in GNB.

A55

Large-Scale Metagenomic Screening Provides Insights into Distribution Patterns of the enigmatic bacterial group Candidate Phyla Radiation (CPR)

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The Candidate Phyla Radiation (CPR) constitutes a vast monophyletic clade within the bacterial domain, encompassing numerous lineages with highly reduced genomes, limited metabolic capabilities, and predominantly epibiotic or parasitic lifestyles. Owing to these atypical traits, CPR bacteria remain largely uncultivated, and amplicon metagenomics studies based on the 16S rRNA gene have proven inaccurate for their detection. Thus, these bacteria have been mostly characterised via shotgun metagenomics. Although numerous studies have highlighted the presence of CPR members in a wide range of ecosystems, their global biome preferences and ecological adaptation patterns remain unresolved.

In this study, we conducted a large-scale analysis of public metagenomic datasets to investigate the distribution and habitat associations of CPR lineages. We developed and applied a machine learning-based classification algorithm using the RecA protein, a core phylogenetic marker, for accurate taxonomic assignment. We applied this method to the MGnify database, which contains bacterial proteins from more than 50,000 shotgun metagenomic samples with annotated biome metadata, enabling us to infer the global distribution patterns of CPR bacteria.

Phylogenetically-aware analyses revealed that CPR bacteria are predominantly found in freshwater environments, with lineage-specific enrichments in wastewater and human-associated microbiomes, suggesting possible host-specific adaptations. Furthermore, analyses at higher taxonomic resolution suggest recent adaptive radiations within several CPR clades.

Overall, we developed a robust bioinformatic pipeline for the detection and classification of bacterial taxa in metagenomic datasets, and applied it to provide novel ecological insights into the diversity and niche specialization of the enigmatic Candidate Phyla Radiation.

Session B - Environmental and industrial microbiology

[Continues from Oral session B. [Click here to view the abstracts from B1 to B10](#)]

B11

β -lactamases from multi resistant Gram-negative bacteria: a molecular target for the discovery of novel inhibitors to be used as β -lactams adjuvants

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Antimicrobial resistance spread represents an enormous global health crisis. Particularly worrying are multidrug-resistant Gram-negative (MDR-GN) bacteria, for whom few therapeutic options are available: β -lactams represent frontline antibiotics, but resistance towards them is widespread. To limit the impact of this largely unmet medical need, in the IN SIGNO project, funded by Lombardy's Fondazione Regionale per la Ricerca Biomedica, we aim at discovering novel antibiotic adjuvants to be used in combination with β -lactams against MDR-GN bacteria. Through the screening of a filamentous actinomycetes/fungi-based microbial library (containing 39,000 crude extracts), several HITs able to restore the activity of β -lactams against five selected MDR-GN clinical isolates were identified. An activity-guided purification process was then applied to isolate the bioactive compounds, which are currently undergoing chemical and biological characterization to proceed with their dereplication, novelty evaluation, and investigation of their mode of action. For this last purpose, the five β -lactamases responsible for the resistance phenotype of the five MDR-GN models used in the screening were heterologously expressed in *Escherichia coli* and purified, most of them at $>5 \text{ mg}_{\text{prot}}/\text{L}_{\text{culture}}$ and with $>90\%$ purity. The recombinant enzymes are currently being characterized (e.g., measurement of steady-state kinetic parameters, determination of protein stability, etc.), as a pre-requisite to use them in *in vitro* and docking assays for studying their interactions with the selected HITs, ultimately contributing to the assessment of their possible preclinical development and future medical use.

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B12 Seasonal dynamics of microbial communities' functions in a WWTP

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Wastewater treatment plants (WWTPs) play a crucial role in the treatment of industrial and urban waters in a city. Activated sludge processes, such as those at Gestione Impianti Depurazione Acque S.p.A. (GIDA) in Prato, Italy, host dynamic microbial communities responsible of ecosystem services such as organic matter degradation and pathogens removal. In this study, we characterized the microbial diversity, metabolic functions and ecological interactions in the sewage, biological denitrification and biological oxidation tanks at GIDA, seasonally over two years.

We found significant taxonomic differences across tanks and seasons, with the sewage tank showing lower diversity and unique community composition. Chemoheterotrophy was the predominant microbial function in the entire WWTP, reflecting the constant need for organic matter degradation. Once removed from analysis, two main functional clusters emerged: nitrogen metabolism and pathogen-associated functions. Nitrogen metabolism was especially active in the sewage tank during winter and spring, primarily performed by *Rhodocyclaceae* and *Comamonadaceae*, which alternated seasonally while maintaining functional stability. Pathogen-related functions were enriched in autumn and summer, particularly in *Burkholderiaceae*, *Acetivibrionaceae*, and *Moraxellaceae*, decreasing in downstream depuration phases.

However, co-occurrence network analysis revealed that functional dominance did not always correlate with ecological centrality. For instance, despite having a high functional relevance, *Rhodocyclaceae* and *Comamonadaceae* showed low connectivity, while less abundant families like *Anaerolineaceae* and *Hyphomicrobiaceae* were highly connected, suggesting structural importance within microbial networks.

Our results highlight the diverse ecological roles of microbial communities in WWTPs, gaining a more comprehensive understanding of microbial ecosystems in such impacted environments.

B13 Educating through microbiome research: a reproducible approach

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Although often unnoticed, the environmental microbiota is closely linked to the health of both the human microbiota and its host. However, its role remains largely misunderstood, especially by non-experts and students. Moreover, collecting environmental microbiota samples (EMS) is time-consuming, requires multiple replicates, standardized metadata, and costly processing and sequencing.

To address these challenges, we developed the *Bicocca Sampling Days* (BSDs), a four-day

event, one day per each season, where students collected EMS across different urbanized areas in the Milano-Bicocca district. Every event started with a lecture and demonstration, continued with sampling and metadata collection with a free data collection, management, and visualization platform and ended with a survey to assess the educational impacts with customized and standard scales. To ensure the reproducibility of this workflow, we provided ready-to-use templates for sampling campaign design, metadata protocols, checklists, and evaluation tools. Over eight hours of fieldwork, 76 students collected 2,429 samples over 29,288.74 m². Participants demonstrated significant gains in microbiome sampling knowledge and confidence in microbiome sampling, with the largest improvements in perceived competence.

To support this model's sustainability, we launched the crowdfunding campaign *Fantastic Microbes and Where to Find Them* to fund EMS sequencing, a free workshop on microbiome data analysis and writing, and public outreach activities that raised awareness and connected citizens with microbiome research.

In conclusion, we offer a reproducible model that integrates microbiome with citizen science, research, and education, while contributing to real-world research and fostering scientific literacy among students and the broader public.

B14 Cd²⁺ and Pb²⁺ bioremediation by a hot-spring isolated strain of *Bacillus subtilis*

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The resistance of bacterial spores to harsh environmental conditions has induced to consider spores as tools for heavy metals bioremediation. Spores of a marine *Bacillus* sp. strain have been reported to efficiently bind manganese (Mn(II)), cobalt (Co(II)) and copper (Cu(II)) ⁽¹⁾, while cells and spores of a *B. coagulans* strain have been shown to efficiently adsorb Pb²⁺ ⁽²⁾. Here, we report that spores of C1, a hot-spring isolated strain of *B. subtilis*, adsorbed Cd²⁺ and Pb²⁺ more efficiently than spores of a lab collection strain of the same species. A comparative genomic analysis of C1 and other strains of the same species is in progress. Metal-adsorption did not alter the structure or function of C1 spores that were still fully resistant and able to germinate after the interaction with Cd²⁺ or Pb²⁺. The spore-adsorbed metals were released upon disruption of the spore coat layers allowing the recovery of the adsorbed metals and suggesting that the metals were accumulated within the spore coat. While Cd²⁺ polluted water impaired a normal germination and growth of seeds of the model plant *Arabidopsis thaliana*, a treatment with C1 spores reduced the levels of Cd²⁺ dissolved in the water and restored germination and growth of *A. thaliana* seeds.

⁽¹⁾Appl. Environ. Microbiol. 1998, 64, 1123-1129

⁽²⁾Chemosphere 2018, 211, 804-816

B15

Invisible Allies: The *Ceratitis capitata* Microbiome Between Insecticide Resistance and Emerging Pathogen RiskC. Baldassarri¹, G. Bettoni², D.M. Minore², F. Piscopiello³, A. Piazza^{3,4}, C. Damiani¹, F. Comandatore²¹*School of Biosciences and Veterinary Medicine, University of Camerino, Italy*²*Department of Biomedical and Clinical Sciences, Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Italy*³*Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Italy*⁴*IRCCS Fondazione Policlinico San Matteo, Pavia, Italy*

Ceratitis capitata, a highly invasive insect pest, poses a significant threat to global food production. It also represents an excellent model for studying tephritid fruit fly invasions worldwide and for developing large-scale integrated pest management (IPM) strategies. Biological control through the Sterile Insect Technique (SIT), although promising and ecologically sustainable, remains limited in large-scale applications. As a result, chemical insecticides such as pyrethroids, spinosyns, organophosphates, and carbamates are still widely used. Recent studies have shown that insect-associated bacteria can play a key role in the development of pesticide resistance. Therefore, characterizing these bacteria is crucial for reducing pesticide usage. Experimental reduction of bacterial communities via antibiotics has demonstrated significant impacts on the physiology and behavior of *C. capitata*, highlighting the microbiota's importance as a potential target in pest control strategies. According to literature, the genera *Klebsiella*, *Enterobacter*, *Pantoea*, *Pectobacterium*, and *Citrobacter* dominate the gut of adult *C. capitata* and other tephritidae. Further, data also indicate enrichment in *Klebsiella oxytoca*, a pesticide-degrading species, and in *Asaia spp.*, recently found to carry a pyrethroid hydrolase gene. In this study, we used shotgun metagenomics to characterize the gut microbiota of *C. capitata* adults from both natural environments and laboratory populations. Preliminary results show the frequent presence of *Klebsiella michiganensis*, an emerging human pathogen carrying plasmids with multiple antibiotic resistance genes, suggesting a close association with the host. Microbial profiles differed significantly between wild and lab samples, supporting the idea that environmental conditions shape microbiota composition and promote microbial convergence in similar habitats.

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B16

Bioprospecting and assessing soil microbiome preservation strategies for sustainable agricultureF. Sbarra^{1,2}, M. Garello³, E. Colantoni¹, F. Sevi¹, A. Visca¹, B. Aracri¹, L. Di Gregorio¹, M. Costanzo¹, S. Tabacchioni¹, F. Aloï³, L. Cocolin³, I. Ferrocino³, C. Varese², D. Spadaro³, A. Bevivino^{1*}*annamaria.bevivino@enea.it¹*Department for Sustainability, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Casaccia Research Center, Rome, Italy*²*Department of Life Sciences and System Biology (DBIOS) (DISAFA), University of Turin, Italy*³*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy*

Soil microbiomes play a pivotal role in ecosystem functionality, particularly in agricultural systems, where they support plant health, nutrient cycling, and disease suppression. Thus, bioprospecting and preserving soil microbiomes are essential strategies for sustainable microbial resource management. The present study aimed to explore and preserve the functional diversity of soil microbial communities, focusing on their potential applications in sustainable agriculture. A combination of culture-based and metagenomic/metabolic profiling techniques was employed to

assess long-term preservation strategies and develop microbiome-based solutions for agriculture. A total of 281 microbial strains were isolated from strawberry rhizosphere and screened for traits relevant to plant growth promotion and disease control. Eight nitrogen-fixing strains were identified, belonging to *Agrobacterium*, *Enterobacter* and *Raoultella* genera. Siderophore production, linked to iron acquisition and plant health, was detected in 109 strains. Antifungal activity against *Botrytis cinerea* was found in 42 isolates, from genera including *Achromobacter*, *Acinetobacter*, *Bacillus* and *Serratia*. To evaluate preservation strategies, rhizosphere soil samples were collected from strawberry plants grown under different field conditions, and grapevine and kiwifruit plants with different phytopathological conditions. Samples were stored at 4°C, at room temperature (freeze-dried), and at -80°C for up 12-months. Functional profiling using the Biolog EcoPlates™ indicated that cryopreservation (-80°C) and refrigeration (+4°C) best maintained microbial metabolic activity, especially in strawberry and kiwifruit soils. While microbiome composition shifted over time, in general -80°C storage most effectively preserved soil microbial communities. These findings support the strategic preservation of rhizospheric microbiomes and the development of multifunctional microbial consortia as biofertilizers for sustainable agriculture.

This work has received funding from “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” SUS-MIRRI.IT project funded by the European Union—NextGeneration EU, PNRR—Mission 4 “Education and Research” Component 2: from research to business, Investment 3.1: Fund for the realization of an integrated system of research and innovation infrastructures—IR0000005

B17

Enhancing Soil Biodiversity and Grain Quality through Organic Agroforestry Rice Cultivation

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Rice is a staple crop globally, yet its conventional cultivation poses significant environmental challenges. Agroforestry offers a more sustainable alternative, enhancing ecosystem resilience—particularly by promoting microbiota biodiversity. In this study, we employed a metabarcoding approach targeting the 16S rRNA gene to characterize the soil microbiota throughout the full production cycle of an organic rice paddy cultivated under agroforestry. Additionally, we analyzed the soil’s chemical-physical parameters and the nutritional profile of the rice grains to identify potential correlations with microbiota dynamics.

Our results revealed significant shifts in microbial community composition over time, with marked increases in overall microbial diversity, peaking during the vegetative and flowering phases of the rice crop. We observed notable species turnover, with distinct microbial communities and indicator taxa associated with each growth stage. Functional analysis indicated a rise in microbial metabolic activity across the production cycle, particularly in pathways related to chemoheterotrophy and the nitrogen cycle. Redundancy analysis (RDA) identified total Kjeldahl nitrogen (TKN) as a primary environmental driver influencing microbial functional composition during the crop’s most active stages.

These findings suggest that organic agroforestry systems foster a dynamic and functionally diverse soil microbiota, contributing to enhanced soil health. Importantly, this cultivation model supports rice production without reliance on chemical inputs such as synthetic fertilizers and pesticides, offering a promising pathway toward more sustainable and ecologically sound agricultural practices.

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B18

Antibiotic resistances and urban parks: impact of urbanization on antimicrobial resistance in soil microbial communities

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The overuse of antibiotics in hospitals, livestock, and agriculture has significantly contributed to the rise of antibiotic resistance, a major global health threat. Antibiotic resistance in these sectors is well studied and monitored, but the resistome in other environments, such as urban park soils, remains poorly investigated. Soil is a key reservoir of antibiotic resistance genes (ARGs), and urban parks, being frequently visited public spaces, may represent an underestimated route of exposure and diffusion.

To address this gap, we analyzed the soil resistome of 10 urban parks in Turin with varying levels of urbanization, that could contribute to the acquisition and spread of ARGs in the natural microbiota. Using an integrated approach combining metagenomics and culturomics, we found that undisturbed (poorly urbanized) environments harbored a higher abundance of efflux-mediated genes, while urbanized parks showed a predominance of ARGs related to antibiotic inactivation, target alteration, and target replacement, mechanisms that were significantly correlated with anthropogenic features. Whereas the enrichment of ARGs in undisturbed parks was likely due to natural competition among microorganisms, as indicated by the observed greater abundance and diversity of fungi, in urbanized parks associations were observed among resistant microbial species and specific environmental factors.

This study highlights the urgent need to include urban soils in One Health surveillance strategies and the importance of monitoring ARGs in urban parks. Promoting sustainable urban design and implementing active soil management are essential to reduce public health risks and support more resilient urban ecosystems.

B19

Development of yeasts for environmentally low-impact ammonia productionA. Pessina^{1,2}, M. Bianchi³, B. Melli¹, G. Bianchi¹, M. Mangiagalli¹, S. Brocca¹, L. Brambilla^{1,2*}*luca.brambilla@unimib.it¹*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy*²*SYSBIO – Centre of Systems Biology, Italy*³*Department of Biology and Biotechnology 'Charles Darwin', Sapienza University of Rome, Italy*

Ammonia (NH₃) is a key chemical compound, essential for agricultural fertilizer production and with growing potential for low-carbon energy storage. Currently, industrial production of ammonia is mainly based on the Haber-Bosch process, an extremely energy-intensive method responsible for a significant share of global CO₂ emissions. However, the ability of certain microorganisms to convert atmospheric nitrogen into ammonia under moderate environmental conditions suggests the possibility of a less energy-intensive and lower-impact production method. We recently demonstrated that the yeast *Saccharomyces cerevisiae* can release significant amounts of ammonia when placed under the right culture conditions without any genome intervention. To explore this phenomenon further, we extended the study to include other related yeasts in an attempt to improve the process. We are currently interested in gene variants of *Kluyveromyces lactis* strains that exhibit the most promising ammonia production. Elucidating the genes involved could help us make this behaviour more predictable, controllable, and generalizable. Moreover, we

identified an additional unconventional yeast strain with excellent performance that could provide a platform for improved metabolic engineering interventions. This strain efficiently transforms nitrogen in soybean processing waste into biomass and ammonia while solving its disposal problems. Developing efficient microbial processes for ammonia production is a promising frontier for the circular bioeconomy because it can valorize carbon- and nitrogen-rich waste streams as fermentation substrates while mitigating the carbon footprint of the fertilizer industry.

B20

Microbial Biodiversity of University Campus and Student Skin: Insights from the UniBiome Project

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Humans spend 90% time in built environments (BE), ecosystems shaped by human activity and architecture, and are exposed to their peculiar microbiomes. Urbanization has profoundly altered both the human microbiome and exposome, yet the relationship between BE and human- α and β -diversities, identified shared core microbiomes, and correlated findings with metadata from participant questionnaires and environmental parameters.

Our analysis revealed high environmental microbiome diversity, encompassing >2000 identified genera. As expected, skin microbiome samples exhibited significantly lower alpha-diversity (Shannon index), with ~800 genera identified. Bray–Curtis dissimilarity revealed distinct indoor and outdoor microbial communities. Indoor environments were dominated by skin-associated taxa, whereas indoor spaces adjacent to green areas supported significantly greater biodiversity and resembled outdoor profiles (core microbiome analysis). Notably, anthropization level of student residences significantly affected skin microbiome composition (Jaccard and Bray–Curtis metrics).

These findings support microbiome-informed urban greening as a means to enhance environmental and human microbiobiodiversity, grounded in the One Health framework.

B21

Belowground Alliances: Effects of Biochar Amendment on Rhizosphere Microbial Communities, Soil, and Grain Yield in Wheat Fields

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This project aims to evaluate the effects of biochar amendment on rhizosphere microbial communities, soil chemical properties, and grain yield in a durum wheat [*Triticum turgidum* subsp. *durum* (Desf.) Husn., variety President] cultivation system.

An experimental field was established in Campobasso city, Molise region, in December 2024. The field was divided into 15 plots of 4 m² each. Five plots received conventional fertilization using organic fertilizers, five plots were amended with biochar, and five plots were left untreated to serve as controls before wheat seeds were sown. Subsequently, rhizosphere soil samples were collected and prokaryotic communities were characterized through Next-Generation Sequencing (NGS) to assess changes in microbial diversity and composition across treatments and controls. In parallel, soil chemical analyses were performed to quantify levels of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals. Sampling campaigns were scheduled for the spring and summer seasons, with the final sampling coinciding with wheat

harvesting and threshing to evaluate the terminal effects of treatments.

The outcomes of this study will provide valuable insights into how biochar amendments can influence the microbial ecology of the rhizosphere, soil chemical features, and crop productivity. The results could support the development of more sustainable and responsible agricultural practices by guiding the application of soil amendments that improve yield while minimizing potential environmental impacts in wheat-based agroecosystems.

B22	Diversity and metabolic potential of cave microorganisms
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Caves are pristine subterranean environments characterized by the absence of light and low amounts of nutrient sources. Cave-dwelling microorganisms survive by establishing cooperation and competition relations that can be associated with the production of molecules/metabolites involved in the modification of rock minerals. Studying the biodiversity and the metabolic potential of cave microbiomes can provide information on the evolution and protection of subterranean environments and might result in the discovery of novel microbes and metabolites with biotechnological interest.

The aim of this work (in the framework of the PNRR-DM118/2023 project) is to explore and compare the microbiology of different gypsum, limestone and quartzite caves to determine the correlation between microbial taxa/functions and environmental parameters and to dig into the microbial roles in different types of subterranean environments. For this purpose, we collected and analysed samples from different caves and we conducted metagenomic analyses, culture isolation and metabolic assays.

The results revealed the presence of diverse microbial communities across different cave environments, exhibiting functional traits related to biogeochemical cycling, rock–microbe interactions, and both cooperative and competitive ecological dynamics. Different bacterial taxa belonging to Actinobacteriota phylum characterized diverse cave systems and exhibited a broad range of enzymatic activities together with the capacity to produce antimicrobial compounds against model pathogenic bacteria.

Overall, this study explores the biodiversity and metabolic potential of cave microorganisms, delving into their ecological roles and evolutionary relevance in subterranean ecosystems.

B23

Characterization of soil microbial communities in high CO₂-enriched sites in the Mt. Amiata geothermal area, ItalyG. Caucia¹, P. Cristiani², S. Ravasi¹, M. Invernizzi³, F. Pittino¹, A. Franzetti¹¹Università degli studi Milano-Bicocca, Department of Earth and Environmental Sciences, Italy²Ricerca sul Sistema Energetico – RSE, Milano (IT)³Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering 'Giulio Natta', Italy

Areas where natural diffuse gases are released typically show CO₂ concentrations in the soil reaching up to 90% by volume—significantly higher than in typical soils. Elevated CO₂ levels have been shown to affect carbon turnover in soil (Beulig et al., 2016), potentially shaping microbial metabolic pathways. Geothermal areas, which naturally emit high levels of CO₂, offer a unique opportunity to study the impact of elevated CO₂ on soil microbial communities. In this study, we monitored gas emissions from key natural sources across different sites in the Mt. Amiata geothermal area (Tuscany, Italy) and assessed how varying CO₂ concentrations influence microbial community composition and function. Water, soil, and biomass samples were collected and analyzed for chemical properties, including pH, carbonates, available phosphorus, organic carbon, and C/N ratio. Microbial community structure (bacteria and archaea) was characterized through Illumina amplicon sequencing and qPCR were performed to estimate bacterial and methanogenic archaeal abundances. Whole genome sequencing will be performed on four samples with high CO₂ concentrations (>94%) to explore microbial metabolic potential. Results showed that high-CO₂ samples were enriched in taxa adapted to acidic, carbon-rich environments, including methanogenic archaea and CO₂-fixing microorganisms. Statistical analyses did not show a significant correlation between CO₂ levels and microbial structure, suggesting a homogeneous and well-adapted community across sites. Notably, *Methanothermobacter*, a thermophilic methanogen with potential applications in Power-to-Gas technologies, was highly abundant. Overall, these findings provide a combined microbiological and geochemical characterization of a geothermal CO₂-emitting area, offering valuable insights into microbial life in extreme terrestrial environments.

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B24

Mechanisms of hexavalent chromium reduction in *Rhodococcus erythropolis* strain SC26L. Cavalca¹, A. Melzi¹, S. Zecchin¹, M. Redondo-Nieto², R. Rivilla²¹Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy²Department of Biology, Universidad Autónoma de Madrid, Spain

Gaining a deeper understanding of bacterial mechanisms involved in the reduction of hexavalent chromium (Cr^{VI}) into less toxic trivalent form (Cr^{III}) is crucial for enhancing bioremediation of metal-contaminated wastewater.

Rhodococcus erythropolis strain SC26 exhibited Cr^{VI} minimum inhibitory concentration of 4 mM. During 145h-growth in the presence of 1 mM Cr^{VI}, it achieves 98% reduction to Cr^{III}. The genome (6.9 Mbp) is composed of one chromosome and four plasmids (two circular and two linear). Although no genes specifically annotated as for chromate reductases are present, several metal resistance-related genes were retrieved: arsenic pump-driving ATPase (*arsA*), zinc import ATP-binding protein (*znuC*), mercuric resistance operon regulatory protein, nickel transport permease (*nikB*), and copper resistance proteins (*copC*, *copD*).

Transcriptomic analyses under Cr^{VI} stress revealed overexpression of pathways for amino acid

biosynthesis, cofactor biosynthesis, carbohydrate metabolism, and ROS scavenging. Real Time q-PCR and enzymatic assays confirmed that NADP-dependent oxidoreductase and glutathione S-transferase are upregulated by Cr^{VI}, suggesting an active role in detoxification and oxidative stress management.

When tested in industrial wastewater, strain SC26 achieved 82% Cr^{VI} reduction, demonstrating its promising potential in bioremediation applications.

This study contributes to the poorly documented Cr^{VI} detoxification mechanisms in *Rhodococcus* genus, identifying key genes and stress-associated regulatory networks, as redox-sensitive transcription factors and stress-response regulators, providing a theoretical basis for future trials in relevant scale Cr^{VI} contaminated environments.

Acknowledgments: PSR 2021 Linea 6 1H_HUB project. A.M. benefits of a UNIMI PhD fellowship - Food Systems PhD Program and Erasmus+ traineeship fellowship.

B25

***Pseudomonas* sp. as a new halotolerant bacterium isolated from contaminated seawater: Efficiency in hydrocarbons biodegradation and biosurfactant production**

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Introduction and objective: Petroleum extraction and industrial activities severely contaminate seawater. Traditional methods for reducing PAH levels are often inefficient, costly, or harmful to the environment. Researchers are, therefore, turning to biotechnological solutions, particularly bioremediation using microorganisms, while addressing the challenges posed by high salt concentrations. In this context, our aim is to isolate and characterize halotolerant hydrocarbon-degrading bacteria and assess their biosurfactant production.

Methods: Enrichment cultures were performed using contaminated seawater from Kraten fishing harbor in Sfax, Tunisia, to screen for aerobic pyrene-degrading bacteria. The growth of the isolated strain on pyrene was monitored *via* optical density at 600 nm and GC-FID analyses. Biosurfactant production was evaluated by measuring surface tension and conducting oil displacement tests.

Findings: A new marine *Pseudomonas stutzeri* strain, PYRKP, was isolated as a pyrene degrader and biosurfactant producer. It degraded approximately 85.1% of pyrene after 7 days and 95.99% after 30 days at 30 g/l NaCl. Strain PYRKP grew on various hydrocarbons, including monoaromatics, PAHs, aliphatic, and complex hydrocarbons. It produced a lipopeptide biosurfactant, named Bios-PYRKP, with a critical micelle concentration of 250 mg/L, reducing surface tension to 33.2 mN/m. Bios-PYRKP demonstrated stability across a wide range of temperatures, salinities, and pH, and effectively removed used motor oil from contaminated soils.

Conclusion: The biodegradation and biosurfactant production potential of the *Pseudomonas* sp. strain PYRKP would present this strain as a favourite agent for bioremediation of hydrocarbon-contaminated sites under saline conditions.

B26

Innovative Antifungal Treatments for the Mitigation of Biodeterioration in Paper-Based Cultural Heritage

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Cellulose-based artifacts stored in archives, libraries and collections are in danger when storage microclimatic conditions are not suitable for their conservation, favouring the growth of biodeteriogenic fungi, which in turns, affects the properties and readability of the valuable paper. In the frame of BIO4ART project, a sampling campaign was carried out in selected rooms of historic libraries, collecting fragments of paper with evident biological alterations and air samples of repository and storage rooms. The approach included: a) qualitative/quantitative isolation of fungal strains, followed by morphological and molecular characterization and evaluation of biodeteriogenic potential through cellulolytic and proteolytic activity assays; b) testing antifungal agents such as boric acid and silver nanoparticles against selected fungal strains by Minimum Inhibitory Concentration (MIC) assays; c) test treatment effectiveness in preventing fungal growth and related biodeterioration phenomena on treated and untreated paper samples inoculated with fungi for up to 40 days. Biocides were evaluated both in pure form and functionalized with crystalline nanocellulose (CNC) derived from cotton waste and oxidized nanocellulose (NC-OX), used as carriers and potential bioconsolidants for paper.

Fungal genera such as *Aspergillus*, *Trichoderma*, *Cladosporium*, were isolated from paper and air samples; 50% of them showed cellulolytic activities, confirming their biodeteriogenic potential. MIC assays demonstrated the effectiveness of boric acid at 3% and silver nanoparticles at concentrations ranging between 15 and 30 ppm. These findings highlight the potential of CNC-functionalized biocides as an integrated approach for preventing fungal biodeterioration and consolidating historical paper documents.

Acknowledgments

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B27

Sub-lethal antibiotic concentrations promote biofilm streamer formation in *Pseudomonas aeruginosa*

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Biofilms, microbial communities embedded in a self-produced extracellular matrix, pose significant clinical challenges due to their role in persistent infections. Extracellular DNA (eDNA) is a crucial biofilm component, contributing to structural integrity and antibiotic resistance. This study investigates how sub-lethal concentrations of antibiotics—commonly used for urinary tract infections—affect the formation of *Pseudomonas aeruginosa* biofilm streamers, filamentous suspended biofilms that grow suspended in flowing environments. Using a microfluidic platform

for controlled real-time observation and with isolated micropillars that trigger biofilm streamer formation, we evaluated streamer development under sub-minimum inhibitory concentration (sub-MIC) exposure to colistin, tobramycin, and norfloxacin. Results reveal that although these antibiotics reduce bacterial surface attachment, they enhance biofilm streamer formation. This increase is driven by eDNA release induced by cell lysis process, which acts as a structural scaffold facilitating streamer assembly and biofilm growth under flow conditions. These findings emphasize the dual role of bactericidal antibiotics in both limiting bacterial attachment to surfaces and promoting eDNA-mediated biofilm development, underscoring the complexity of bacterial responses to suboptimal antibiotic treatment. Understanding these dynamics is critical for improving antibiotic strategies to combat biofilm-associated infections effectively.

B28

Analysis of a tellurite resistant mutant to explore *Rhodococcus* stress adaptation and resistance strategies to toxic metal(loid)s

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Bacteria belonging to the *Rhodococcus* (*R.*) genus are known for their remarkable ability to tolerate and transform toxic metal(loid)s, including tellurite TeO_3^{2-} , a highly reactive and toxic oxyanion. These properties make *Rhodococcus* strains promising candidates for applications in environmental biotechnology, such as the remediation of polluted sites, the biosynthesis of metal(loid)-based nanostructures for industrial or biomedical use, and the recovery of rare and valuable elements. *R. aetherivorans* BCP1 has been shown to convert toxic tellurite into elemental tellurium (Te^0), forming metal nanostructures that are less toxic than the oxyanion and recoverable from the environment. However, the underlying molecular mechanisms driving this resistance and transformation are still poorly understood. This study aims to characterize the cellular response to tellurite stress in *Rhodococcus*, comparing the wild-type strain BCP1 (BCP1 WT) with a spontaneous mutant (BCP1-Wh) isolated under high tellurite concentrations. The mutant exhibits a white phenotype due to a frameshift mutation in the *ctrl* gene, encoding phytoene dehydrogenase, an enzyme involved in carotenoid biosynthesis. Carotenoids are known to play protective roles against oxidative stress and to support membrane integrity. Physiological assays revealed that BCP1-Wh has greater tellurite tolerance than BCP1 WT, with enhanced planktonic and biofilm growth. No major differences were observed in tellurite uptake or ROS levels, but BCP1-Wh showed increased resistance to external oxidative agents like hydrogen peroxide, indicating the activation of alternative defense mechanisms. Ongoing comparative multi-omics analyses will further elucidate these adaptive responses, contributing to our understanding of microbial strategies for metal(loid)s detoxification, with potential applications in bioremediation and sustainable metal recovery.

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B29**Microbial community diversity in Levante Bay during the 2022 increased volcanic activity in Vulcano Island**

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A major hydrothermal unrest was observed on Vulcano island (Aeolian archipelago, Italy) in 2021-2022. The substantial influx of volatiles (*i.e.*, CO₂, SO₂ and other minor species), released by degassing magma at depth strongly altered the physico-chemical conditions of the shallow hydrothermal system underneath Levante Bay, north of the main active (La Fossa) cone. This perturbation led to a significant increase in the partial pressure of CO₂. From 2007 to 2021, total soil CO₂ output ranged between 1.2 t d⁻¹ and 16.4 t d⁻¹, with a clear increasing trend observed from 2015 onward (Inguaggiato et al., 2022). Sciutteri et al. (2022) demonstrated that geochemical conditions and physico-chemical parameters, specifically oxido reductive potential and pH were the primary drivers shaping the composition and metabolic potential of microbial communities in Levante Bay. The aim of this study is to describe the diversity of microbial communities under such increased volcanic activity. To do so, sediment samples were collected in September 2022 from locations at increasing distance from the main hydrothermal vent in Levante Bay. Samples were subjected to shotgun metagenomic sequencing to investigate the functional and taxonomic diversity of the community. More than 200 Metagenome assembled genomes (MAGs) were reconstructed and annotated. The analysis revealed that samples differentially exposed to the hydrothermal fluid show distinct taxonomic and functional profiles, clearly indicating a response of the microbial community to the increased volatile flux. This study highlights strong interplay between geological processes and biological responses that underpin the coevolution of life with our planet.

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B30**Biotechnological potential of mosaic isolated strains and local products for restoration: antimicrobial properties of formulations based on microbial biosurfactant-containing solutions and essential oils**

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The conservation of Cultural Heritage (CH) is a key issue to preserve socioeconomic resources but works of art face constant threats from deterioration. Natural stones are subject to physical, aesthetic, and chemical damage caused by the growth of biodeteriogens and the extracellular polymeric substances they produce. However, not all CH-inhabiting microorganisms are biodeteriogens: some have biocleaning potential for the antimicrobial and antibiofilm properties of

the biosurfactants they produce. Essential oils display similar activity and are gaining interest in conservation for their effectiveness and attainability.

The metabolites of two mosaics' bacterial isolates and locally sourced essential oils were tested for antimicrobial/antibiofilm activity. The physico-chemical alterations and antifungal effects of formulations based on microbial biosurfactant-producing strains and essential oils were tested on marble.

The tested formulations of microbial biosurfactant-producing strains (*Bacillus subtilis* and *velezensis*) and essential oils showed antimicrobial/antibiofilm activity against human pathogens and microbial strains from the same mosaics. Their application on marble does not cause surface alterations in terms of colour, pH, and peeling, but slightly changes its wettability. Preventive and cleaning effects on white marble colonized by *Aspergillus niger* produce reduction of ATP activity, delayed sporulation, and minimal proliferation, with reduced colour modification, pH neutralization, and no stone peeling.

Using microbial biosurfactants as alternatives to synthetic ones ensures sustainability, environmental stewardship, and circular bioeconomy applications. Their combination with essential oils enhances antimicrobial, antibiofilm, and emulsification properties. Formulations based on *Bacillus* strains and essential oils may be a promising base for developing green products for CH conservation.

B31

Bioregeneration of contaminated carbonaceous filters applying top-down and bottom-up synthetic biology approaches

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Native bacteria living in contaminated groundwaters, as well as those colonizing filtration systems used to remediate them, possess the genetic and metabolic potential for the recovery of end-life carbonaceous filters, such as activated carbons (ACs) and biochar. Biochar is an adsorbent material produced via pyrolysis of organic biomass under oxygen-limited conditions and is studied as possible sustainable and low-cost alternative to ACs. Our work aimed at designing and applying synthetic microbial consortia and evolved communities to remove contaminants from spent filters in the framework of circular economy. A total of seventy bacterial strains were isolated from water and filters samples collected from two groundwater treatment plants characterized by different types of contamination, aromatic hydrocarbons and ethers, or chlorinated compounds. Two synthetic consortia were constructed by selecting bacterial strains based on their metabolic capabilities to grow on different contaminants and testing their compatibility. In parallel, Adaptive Laboratory Evolution (ALE) experiments were performed using contaminated waters and exhausted filters to generate evolved communities optimized for the degradation of the desired adsorbed contaminants. GC-FID analyses were conducted to evaluate the capacity of synthetic consortia and evolved communities to bio-regenerate the filters. As a result, we observed a better performance of contaminant removal on biochar filters as compared to ACs, together with a higher removal of aromatic hydrocarbons as compared to chlorinated compounds. In conclusion, both synthetic microbial consortia and evolved communities can be applied to remove contaminants from end-life filters, enabling their potential re-use in other bioremediation applications.

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B32	Alien invaders and microbial rewiring: how <i>Carpobrotus</i> spp. shape soil microbial diversity and how native microbiota might turn the tide
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Carpobrotus spp. are known to alter vegetation and soil in Mediterranean habitats, but their impact on other communities remains understudied. We adopted a multi-taxa approach to assess the ecological effects of *Carpobrotus* on Giglio Island (Italy), focusing on soil microbial communities and testing strategies for soil fertility recovery.

Rocky garrigue habitats were surveyed across 18 plots categorized as invaded (I), eradicated from previous invasion (E), and natural (C). We collected data on vascular plants, bryophytes, ants, soil microarthropods, molluscs, and microbiota, alongside abiotic soil parameters. Microbial communities were characterized via targeted metagenomics.

Carpobrotus invasion caused marked shifts in microbial diversity and community structure, paralleling vegetation and soil changes. One year after eradication, soils showed ruderal colonization and altered traits. To test microbiome restoration, we inoculated native microbial consortia (NMC) from C soils into I and E soils, monitoring the germination and growth of *Cistus* (native shrub) and *Medicago sativa* cv. Marina (salt-tolerant model). We also evaluated plant growth-promoting (PGP) traits of the inocula. NMC inoculation improved *Cistus* germination in both I and E soils, suggesting that key microbial functions can be restored even in degraded sites. *Medicago* showed enhanced growth, though not germination, possibly due to specific microbial dependencies. PGP analysis revealed higher production of biosurfactants and auxins in NMC from C soils compared to I and E.

Our results highlight the deep impacts of *Carpobrotus* on soil microbiota and suggest that microbiome-based interventions could support the recovery of functional biodiversity in Mediterranean ecosystems.

B33	The MYCOSEAS Project: “Production and characterization of new bioactive molecules against emerging and/or multidrug-resistant pathogens by neglected poly-extremophilic marine fungi”
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Microbial diseases with unavailable treatment cause many medical issues; moreover, increasing of social/medical concerns are associated to emerging pathogens, including new or scarcely studied microbes. Besides, increasing problems are due to known antibiotics-resistant and/or biofilm producing microorganism. Clinical microbiology constantly requires new therapeutic strategies and molecules. Marine environment has been somehow underevaluated for the discovery of new strains exploitable for novel bioactive molecules. Marine fungi represent a diversified source of bioactive metabolites for new drugs development. Neglected species need to be carefully studied for new bioactive molecules production. In the Mycoseas project

selected marine fungi, are investigated for their production of new secondary metabolites (SMs) active against emerging pathogens, also related to biofilm matrixes. Thirty strains, from new or neglected species, had been chosen and screened for high SMs production, targeting secondary metabolism genes (NRPS, PKS, TS), allowing to select 10 strains for subsequent steps. To stimulate the expression of silent SMs genes, the strains had been cultivated on various solid and liquid media under different chemico-physical growth conditions (OSMAC). Culture extracts had been tested for their antiviral, antimicrobial and antibiofilm activity, and active extracts by metabolomic techniques to get a metabolic network relating metabolite production and culture conditions. For best promising strains, SMs production will be upscaled for further activity tests and bioactive molecule characterization. A library of enriched positive fractions will be used for the activity tests. Isolation/purification of bioactive compounds will allow to determine their chemical structure. Purified molecules will be tested for their biological activity too.

The project is financed by the E.U “Next Generation EU” by the PRIN 2022 framework (Project n°: 2022MPTT35)

B34

Mycoaugmentation shapes microbial community structure and function in historically petroleum-contaminated clay-rich soil: A genome-centric perspective

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**These authors contributed equally to this work*

Bioremediation of clay-rich soils contaminated with petroleum hydrocarbons presents significant challenges due to the strong adsorption of hydrocarbons and limited oxygen availability.

In this study, we investigated the effectiveness of mycoaugmentation in enhancing petroleum hydrocarbon removal from a historically contaminated, clay-rich soil containing 825 mg/kg of C_{12} and 6,990 mg/kg of C_{12} – C_{40} hydrocarbons (soil dry weight). After evaluating hydrocarbon degradation and phytotoxicity reduction by nine fungal strains encompassing different species, a genome-centric approach was adopted to characterise the microbial community in the most effective mycoaugmented microcosm. In particular, microcosms augmented with *P. ostreatus* P24 were analysed to assess microbial composition and metabolic potential, and to determine whether hydrocarbon-degrading bacteria were selectively enriched compared to the incubation control (AIC) amended only with wheat straw and alfalfa hay.

The microbial community associated with AIC and P24-augmented microcosms exhibited time- and treatment-dependent changes, suggesting that the fungal inoculum shaped the microbial community structure. Notably, as a consequence of fungal-driven lignocellulose breakdown, novel lineages of nitrogen-fixing and polysaccharide-degrading Clostridia were enriched explicitly in P24-augmented microcosms, supporting potential mutualisms between fungi and diazotrophic bacteria. The alkane-degrading population also shifted over time and between treatments. The reduction of long-chain hydrocarbons measured in AIC and P24-amended microcosms was accompanied by a significant increase of species carrying long-chain alkane monooxygenases (*ladA*), with a specific enrichment of *Actinotalea* and *Azorhizobium* in the mycoaugmented microcosms. These results highlight potential inter-kingdom interactions between hydrocarbon-degrading bacteria and the fungal inoculum, which may be exploited to enhance petroleum hydrocarbon removal.

Funding

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B35

Pomegranate Extracts from Agri-Food Waste: *In Vitro* Antifungal and Antibiofilm Effects on Emerging *Candida* Pathogens

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Infectious diseases caused by *Candida* species remain a significant cause of morbidity, particularly among immunocompromised individuals and hospitalized patients. The pathogenicity of these yeasts is attributed to several virulence factors, including morphological switching, germ tube formation, and the development of biofilms. Biofilms, particularly, contribute to increased resistance to antifungal agents and host immune defenses, making biofilm-associated infections especially difficult to treat. This clinical challenge underscores the urgent need for alternative, effective, and sustainable antifungal strategies. In this context, agri-food waste has emerged as a valuable reservoir of bioactive compounds with therapeutic potential. This *in vitro* study investigated the antifungal activity of a polyphenol-rich extract obtained from the peel of *Punica granatum* L. (pomegranate) against conventional and emerging *Candida* species. The hydroethanolic extract of pomegranate peel was assessed using both qualitative and quantitative methods to determine its antifungal efficacy and its ability to interfere with key virulence traits, such as germ tube formation and biofilm development. Clinical isolates of *C. albicans*, *C. glabrata*, and *C. parapsilosis* were employed in the assays. The results demonstrated that the extract exhibited notable antifungal activity, exerting both fungistatic and fungicidal effects across all tested strains. Furthermore, the extract effectively inhibited germ tube formation and biofilm development, including the prevention of initial fungal adhesion and the disruption of established biofilms. These findings provide valuable insight into the multi-targeted antifungal potential of pomegranate peel extract, suggesting its promise as a natural, sustainable approach to combat *Candida*-related infections—particularly those involving biofilm-related resistance.

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B36

How genome-scale metabolic models and metabolomics help understand the functional role of microbes and viruses in salt marsh sediments

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Tidal salt marshes with their peaty soils, halophilic plants and large chemical-physical fluctuations act as biodiversity and biogeochemistry hotspots. The worldwide loss of salt marshes, caused by erosion, storm surges, rising sea levels, and human activities, calls for multidisciplinary research and new metrics enabling their conservation, restoration and construction. Focusing on natural and restored salt marsh areas in the Venice Lagoon, we characterised abiotic and biotic components of surface sediments, integrating data from Genome-scale metabolic models (GEMs), sediment metabolome and virus-encoded genes, to pinpoint key processes and ecosystem services. GEM analysis and sediment metabolite profiles outlined the fermentative and aerobic utilisation of

multiple carbon sources and a balance between catabolism and anabolism. The predicted virus hosts largely mirrored prokaryote taxonomy. Moreover, GEMs and annotated virus genes outlined the influence of phages on pentose phosphate and amino-sugar pathways, redox metabolism and lipopolysaccharides biosynthesis. Our results point to the competence of the microbial community for the degradation of complex organic matter, maintenance of metabolite pools, detoxification of fermentative intermediates and products, and highlight the importance of multidisciplinary approaches for microbiome investigations.

This study is mostly funded by NextGenerationEU-PNRR-M4C2 Investments 1.4 (NBFC CN_00000033-C93C22002810006) and 1.5 (iNEST ECS00000043-C43C22000340006).

B37**Microbial and Bioactivity Profiling of Avocado Fruits: Unlocking Novel Biotechnological Potentials**

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Avocado is a widely consumed tropical fruit renowned for its exceptional nutritional profile and diverse range of biological activities. These include antimicrobial, antioxidant, antifungal, and plant growth-promoting (PGP) effects, which contribute to its health benefits and underscore its potential in various biotechnological applications. Recent scientific investigations have highlighted that many of these bioactivities may be closely linked to the rich and diverse microbiota associated with different parts of the fruit. The endophytic microbial communities inhabiting the avocado peel, pulp, and seed may play crucial roles in modulating the fruit's biochemical landscape and enhancing its functional properties.

Driven by increasing global demand, avocado cultivation, production, and consumption have grown rapidly in recent years. This has led to the generation of considerable amounts of agricultural waste, primarily in the form of seeds, peels, and defatted pulp. These by-products, which make up around 30% of the total fruit mass, remain largely underutilized despite their potential as valuable sources of microorganisms and bioactive metabolites.

This project aims to comprehensively characterize the microbiota of avocado peel, pulp, and seed using both culture-dependent methods and culture-independent metataxonomic approaches based on next-generation sequencing (NGS). The objective is to identify key microbial taxa and isolate novel strains associated with beneficial biological functions. In parallel, extracts from each fruit component will be evaluated in vitro for bioactive properties to identify new metabolites with potential biotechnological applications in agriculture, food preservation, and biomedicine. The integration of microbial and metabolite profiling will provide insights into the ecological and functional roles of avocado microbiota, supporting the sustainable valorization of avocado by-products.

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B38

Microbial taxonomic and functional diversity across the Drake Passage and the west Antarctic Peninsula

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Antarctica and the Southern Ocean are central to the Earth's climate and oceanic circulation systems. Microorganisms inhabiting these water bodies drive biogeochemical cycles and underpin trophodynamics. The ongoing climate crisis is affecting these pathways in the Southern Ocean, with possible cascading effects on the structure and functioning of the microbial communities of surface ocean waters. However, the diversity and distribution of marine microorganisms in the Southern Ocean are still poorly resolved, and microbial diversity analysis along large scale transects in Polar regions are lacking compared to temperate and tropical regions.

Previous studies on microbial communities in the surface waters of the west Antarctic Peninsula and in the Southern Ocean have reported a dominance of Gammaproteobacteria, Alphaproteobacteria and Bacteroidetes, and exhibit metabolic functions related to degradation of phytoplankton derived organic matter. Here, we present data on the microbial diversity of surface waters along the Drake Passage and the west Antarctic Peninsula, sampled during the 2023/24 Austral Summer, combining shotgun metagenomic and 16S rRNA amplicon analysis with chlorophyll chemotaxonomy and geochemical analyses. Our results reveal changes in community structure associated with shifts in oceanic currents and dominant phytoplankton groups. By integrating taxonomic and functional microbial diversity with geochemical analysis, this work will enable to advance our understanding of microbial responses to the impacts of climate change. It also contributes to identifying specific microbial pathways in the Southern Ocean, ultimately helping to fill gaps in climate change modeling.

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B39 Analysis of the microbial communities of the Adamello Glacier

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Global warming has led to a drastic reduction of glacier volumes, with impacts on water resources, hydrogeological risk, sea level rise, and biodiversity.

The supraglacial environment is influenced by solar radiation and atmospheric inputs and is the most biodiverse glacial environment. It hosts complex microbial communities, with phototrophs and chemosynthetic bacteria as the primary producers.

This study aimed to characterize the microbial communities of different supraglacial habitats (i.e. cryoconite, dirt cones, debris, and surface ice) of the Mandrone Glacier (the main tongue of the Adamello Glacier, central Italian Alps) from samples collected in July and August 2023 through amplicon sequencing (Illumina platform).

In particular, bacterial and fungal communities were characterized by sequencing the V5-V6 regions of the 16S rRNA gene and the ITS region, respectively. The sequences obtained were then grouped into ASVs and classified using the RDP and UNITE databases for bacterial and fungal communities respectively. At phylum level, the most abundant bacteria belong to Proteobacteria, Actinobacteria, Cyanobacteria, and Bacteroidetes, while among fungi Ascomycota, Basidiomycota, Mucoromycota, and Chytridiomycota prevail.

Multivariate analyses showed significant differences in microbial composition in relation to the sampling time and habitat. Variation partitioning showed that, for bacteria, the month explained a greater share of the variance than the habitat, while for fungi the influence of the environment prevailed.

These results contribute to enriching the knowledge of the microbial biodiversity of the largest glacier in the Italian Alps, providing essential data to understand the ecological dynamics of this ecosystem subjected to rapid changes.

B40 Microbial Hitchhiking as a Strategy for the Targeted Delivery of Spores to the Rhizosphere

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Microbial hitchhiking is a newly recognized ecological strategy by which non-motile microorganisms exploit the motility of other microbes to enhance their spatial dispersal. In this study, we investigated the ability of *Bacillus subtilis* spores—dormant, stress-induced forms—to attach to the flagella of motile bacteria. We focused on *B. subtilis* strains isolated from both soil and the human gut, as well as on other motile bacterial species. Using a collection of motility-deficient mutants—including strains lacking flagella or impaired in swarming behavior—together with reporter genes such as *lacZ* and *gfp*, our preliminary assays demonstrated that spore adhesion is dependent

on the presence of functional motility systems in the host bacterium. These findings suggest that spores may utilize this hitchhiking mechanism to escape unfavorable microenvironments and reach niches more conducive to germination and growth. Beyond its ecological relevance, this mechanism holds promising potential for applications. In particular, it could be harnessed as a natural delivery system for transporting effector molecules—such as biofertilizers—directly to plant roots, thereby supporting more sustainable and environmentally friendly agricultural practices.

B41

Marine species belonging to a new *Tamaricicola* lineage with promising antiviral potential

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Marine fungi are a largely unexplored component of marine biodiversity, with a significant potential for ecological and biotechnological applications. As a part of a survey of fungal diversity in the Tyrrhenian Sea, six fungal strains were isolated from the seagrass *Posidonia oceanica* and the jellyfish *Pelagia noctiluca*. These strains were characterised using a polyphasic approach, integrating morphological, physiological, and molecular data. Morphological analyses were performed on various cultural media and under different conditions to promote the production of reproductive structures. The strains exhibited characteristic globose ascomata with a thick-walled peridium, bitunicate cylindrical asci with muriform ascospores, and a coelomycetous anamorphic phase characterised by conidiophores that were reduced to phialidic conidiogenous cells, which produced ellipsoidal, hyaline, single-celled conidia. Additionally, muriform chlamydospores were identified. Molecular phylogenetic inference, based on a concatenated dataset of five markers (nrSSU, nrITS, nrLSU, *tef-1α*, and *rpb2*), confirmed that the isolates belonged to a new lineage within the currently monospecific genus *Tamaricicola*. Based on these findings, the new species *Tamaricicola fenicis* sp. nov. is proposed. Given the known biotechnological relevance of marine fungi, the strains were also screened for the production of bioactive secondary metabolites. Notably, the CHCl₃ extract of *T. fenicis* PN38 exhibited strong antiviral activity against Echovirus 11 (Enterovirus), achieving 100% inhibition at 50 µg/mL. These results suggest its promising potential in antiviral drug discovery and warrant further investigation.

The project is financed by the E.U “Next Generation EU” by the PRIN 2022 framework (the MYCOSEAS Project; n°: 2022MPTT35)

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B42	Urban Phyllosphere Microbiomes as Agents of Air Pollutant Biodegradation: A Case Study from Milan, Italy
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Urban plants and their associated phyllosphere microbiomes hold potential for the biodegradation of airborne pollutants such as polycyclic aromatic hydrocarbons (PAHs) and alkanes, which originate mainly from anthropogenic sources and accumulate on leaf surfaces. Although phyllosphere microbes are known to degrade such compounds, the ecological factors shaping their composition and functional capabilities remain insufficiently explored.

In this study, we investigated microbial communities from the leaves of four plant species and from bioaerosols, sampled across three locations in Milan (urban traffic area, peri-urban park, and extra-urban park) and four seasons. Amplicon sequencing of the 16S rRNA gene and ITS1 region was performed to assess microbial diversity. These data were then analyzed in relation to environmental variables, including concentrations of PM10 and PAHs.

To estimate biodegradation potential, relative abundances of bacterial genera were combined with functional coefficients (ranging from 0 to 1), calculated based on the proportion of genomes in the GTDB database containing hydrocarbon-degrading genes, as identified using Hidden Markov Models (HMMs).

Microbial community composition was significantly influenced by plant species, season, and location, including interactions among these factors. Several bacterial genera with potential to degrade long-chain alkanes and aromatic hydrocarbons were detected.

Overall, the results highlight the combined influence of ecological and environmental factors in structuring phyllosphere microbial communities and shaping their biodegradation potential. This study supports the role of phyllosphere microbes as contributors to urban air pollutant degradation and lays the groundwork for future strategies in microbiome-informed phytoremediation.

Acknowledgements

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References

Franzetti, A., Gandolfi, I., Bestetti, G., Schioppa, E. P., Canedoli, C., Brambilla, D., ... & Ambrosini, R. (2020). Plant- microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of hazardous materials*, 384, 121021

B43 Repurposing the old drug clofoctol as colistin adjuvant

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Colistin is a last-resort antibiotic used to treat infections caused by Gram-negative pathogens. Despite concerns about its nephrotoxicity, its clinical use has increased due to an alarming rise of multidrug resistant (MDR) bacterial strains. However, the emergence of colistin resistance in various clinical settings worldwide underscores the urgent need for colistin adjuvants.

We recently demonstrated that clofoctol, an FDA-approved synthetic antibiotic active against Gram-positive bacteria, restores colistin susceptibility in clinically relevant colistin-resistant ESKAPE pulmonary pathogens, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

In this study, whole-genome sequencing of *P. aeruginosa* mutants resistant to the combination treatment, as well as computational molecular dynamics simulations (CMDs) and spectrophotometric binding analyses, were used to investigate the mechanism underlying the synergistic activity of the colistin-clofoctol combination, using *P. aeruginosa* as a model organism. Mutants resistant to the combination emerged at very low frequency. Genomic analysis revealed mutations associated with resistance to both the combination and colistin alone, indicating that resistance arises via alterations in colistin rather than clofoctol targets. Binding assays showed a 1:1 interaction between clofoctol and colistin. CMDs suggests that clofoctol stabilizes colistin in a conformation favorable for interaction with the bacterial outer membrane.

In conclusion, we propose that clofoctol functions as a molecular chaperone, enhancing colistin bactericidal activity by stabilizing it in its active conformation. Further studies are underway to validate this hypothesis and assess the potential clinical utility of the colistin-clofoctol combination.

B44 Chatting at the Extremes: How Quorum Sensing Changes Across Extreme Environments

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Quorum sensing (QS) is a sophisticated cell-to-cell communication mechanism used by microorganisms to sense their environment and coordinate collective behaviors. This system relies on the production, release, and detection of specific extracellular signal molecules, which accumulate according to population density. Once a critical threshold concentration is reached,

these signals trigger coordinated gene expression across the microbial population. In natural context, QS is widespread and can affect a broad range of biological processes, including nutrient cycling, biofilm formation, host-microbe interactions, and environmental adaptation. Several QS systems have been identified, each relying on specific signal molecules and pathways. This diversity carries important implications, as certain signaling molecules exhibit greater stability than others under specific environmental conditions.

In extreme environments, where harsh conditions push life to the edge of its survival limits, microbes are often the only form of life capable of surviving and thriving. Although still poorly understood, it is possible that QS plays a crucial role in regulating their adaptive responses to environmental stressors.

This study aims to describe patterns in the distribution of QS genes in diverse extreme environments. We annotated a QS-related proteins database with KEGG Orthology (KO) identifiers and compared them to KO profiles from our metagenomic dataset, comprising samples from over 300 extreme sites worldwide; these latter were also characterised through high resolution geochemical analyses. Overall, this comparison allowed us to evaluate the presence, distribution, and relative abundance of QS-related functions across different environmental and geochemical contexts.

B45**Switching to eco-friendly products: exploring innovative solution for a sustainable disinfection in health care environments**

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Healthcare-associated infections (HAI) are a global problem, and studies suggest a direct correlation between surface contamination and HAI. Control of contamination has so far been addressed using chemical-based sanitization procedures, which however have limitations, as evidenced by the persistence of contamination itself and the increasing antimicrobial resistance (AMR) in microorganisms that cause HAI. Recent studies demonstrate that more than 50% of surfaces are not adequately decontaminated and several microbes persistently reside on treated surfaces leading to a selection of AMR strains. Given the rapid increase in multidrug-resistant bacteria and the growing awareness of the importance of environmental contamination, the aim of the study is to develop a new effective sanitization system combining natural compounds with some more commonly used biocides. Co-exposure to biocides and natural substances aims to increase the targets on the bacterial cell and, consequently, to obtain both a bactericidal effect and a low risk of inducing resistance. The efficacy of individual natural compounds, chemical biocides and their mixtures was determined using minimum inhibitory concentrations and checkerboard methods against planktonic microorganisms and 2-hour adherent bacteria. Finally, possible changes in the presence of virulence factors and genes conferring reduced susceptibility to biocides in the tested strains were evaluated following exposure to biocides, phytochemicals and mixtures. The aim is both to improve the quality of cleaning practices to reduce environmental contamination by antimicrobial resistance in hospitals and to counteract the selection of resistant strains through the use of chemical biocides.

The study was supported by FAR2024PD grant to Ramona Iseppi.

B46

Towards the Biocontrol of Rice Foot Rot: Genotypic and Phenotypic Characterization of Novel Bacteriophages Targeting *Dickeya oryzae*L. Magnani^{1,2}, I. Bertani¹, C. Bez¹, A. Bonacorsi², C. Campobasso², V. Venturi^{1,3*}, M. Di Luca^{2*}¹Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy²Department of Biology, University of Pisa, Italy³African Genome Center, University Mohammed VI Polytechnic (UM6P), Ben Guerir, Morocco

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Phage therapy is gaining renewed attention for treating drug-resistant bacterial infections in clinical and veterinary settings. It is also emerging as a promising biocontrol strategy for managing bacterial diseases in agriculture, as current treatments still rely largely on broad-spectrum antimicrobials. These often affect non-target microorganisms and drive resistance, highlighting the need for more precise, sustainable alternatives.

This study aimed to isolate and characterize bacteriophages targeting *Dickeya oryzae*, a phytopathogenic bacterium responsible for rice foot rot disease, an emerging threat to rice cultivation, with cases reported across Asian countries and in Italy.

Following enrichment from environmental samples, three phages were isolated. To our knowledge, these are the first bacteriophages reported to infect *D. oryzae*. Genomic characterization was performed via Illumina paired-end sequencing, and host range was assessed by spot assay. *In vitro* lytic activity was evaluated over 24 hours, both individually and as a three-phage cocktail, at Multiplicities Of Infection (MOIs) from 0.01 to 100.

Two of the phages are jumbo phages (genome >200 Kbp), both representing novel species, with one also defining a new genus. The three phages exhibit complementary host ranges, together covering most of the tested strain collection. In infection kinetics assays, while individual phages at certain MOIs did not effectively reduce bacterial growth, the cocktail significantly suppressed proliferation, suggesting enhanced efficacy through combination.

Ongoing experiments are assessing phage efficacy *in planta*, with encouraging preliminary results. These findings support the high potential of phages and phage cocktails as biocontrol tools for managing *D. oryzae* in agriculture.

B47

Leaf surface traits influence *Salmonella enterica* attachment in green salad varietiesS. Truschi¹, L. Marini¹, I. Cacciari², A. Baldi¹, P. Bruschi¹, A. Lenzi¹, J. Baales³, V.V. Zeisler-Diehl³, L. Schreiber³, M. Marvasi⁴¹Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Italy²CNR, Institute of Applied Physics 'Nello Carrara', Sesto Fiorentino, Florence, Italy³Institute of Cellular and Molecular Botany (IZMB), University of Bonn, Germany⁴Department of Biology, University of Florence, Italy

The attachment of human pathogens to the plant phyllosphere is one of the first steps in produce contamination. Research has shown that the leaf surface plays a crucial role in bacterial adhesion. Surface roughness creates microhabitats—such as fractures, pores, and irregularities—that allow bacteria to anchor and shelter from environmental stressors like wind, rain, and UV radiation. The chemical makeup of the leaf surface, including the cuticle and waxy layers, also influences attachment. Waxes, especially those forming the epicuticular layer, may enhance bacterial adhesion by providing favorable surface properties.

This presentation summarizes recent findings from a multidisciplinary study examining the attachment of *Salmonella enterica* on the leaves of 30 green salad varieties. The study focuses on how specific leaf traits—such as surface roughness, wettability, epicuticular waxes, and water status—affect susceptibility to *Salmonella* contamination.

Understanding these interactions is key to improving food safety in leafy greens. Identifying traits that influence pathogen attachment can inform breeding programs aimed at developing less susceptible cultivars. These insights are valuable not only for researchers studying plant–microbe interactions but also for producers, processors, and food safety professionals working to reduce contamination risks in the fresh-cut industry. Ultimately, this knowledge supports the development of safer salad greens from farm to fork.

B48

Microbial dynamics and MCFAs production from organic waste in a fixed-bed biofilm reactor

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In a circular economy approach, organic waste can be valorized to produce high-value compounds. Among the first applications, this study aimed to promote medium-chain fatty acids (MCFAs) production from organic waste in a fixed-bed biofilm reactor.

Lab scale fixed-bed biofilm reactors filled with sintered glass rings were inoculated with fermentative biomass and operated under mesophilic anaerobic conditions; multiple feeding strategies were tested using different substrates, including food waste and waste activated sludge. Control tests were conducted under the same operating conditions without the addition of filling material.

Overall the presence of filling material enhanced the production of MCFAs and improved yields in biofilm-based systems under all tested conditions, reaching a maximum concentration of 157.6 gC₆/gV_{S_{red}}.

In biofilm samples, the application of in situ hybridization techniques, 16S rRNA gene sequencing (Illumina and Nanopore methods), and quantitative PCR (qPCR) revealed an enrichment of *Firmicutes* (i.e., *Caproiciproducens*, *Pseudoramibacter*), which are known for their slow growth rates and high capability to produce MCFAs. In contrast, the liquid phase was characterized by a higher abundance of *Actinobacteria* (i.e., *Olsenella*), with a rapid fermentation kinetic. The abundance of *BCoAT* gene (key functional gene for the MCFAs production) assessed with qPCR, was higher in biofilm-associated communities, in line with the highest MCFAs production observed in the biofilm reactors compared to the control tests.

This study demonstrated the potentiality of biofilm reactors for the production of MCFAs from organic waste, thus providing crucial hints for improving production yield and paving the way for new circular economy strategies.

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B49 Antibiofilm properties of polyphenols under static and dynamic conditions

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Biofilm-forming microorganisms adhere to surfaces and are embedded in an extracellular polymeric matrix, making them up to 1,000 times more resistant to antibiotics than planktonic bacteria. This presents a major health and economic challenge worldwide. This study investigates the antibiofilm efficacy of natural polyphenol-rich extracts (PREs) as potential alternatives for preventing or reducing biofilm-associated infections.

Clinically relevant strains such as *Pseudomonas aeruginosa* were cultured in growth medium and incubated for 24 hours at 37 °C in 96-well plates with different concentrations of polyphenols from natural sources to assess their effect on planktonic growth. Surface-associated growth under fluid shear was also studied in PDMS microchannels at flow rates from 1 to 5 µl/min and at 37 °C. Surface colonization and biofilm formation were monitored via phase-contrast and fluorescence microscopy, employing fluorescently labeled lectins to visualize the extracellular matrix.

Polyphenol-rich extracts showed significant antibacterial activity under static conditions by inhibiting bacterial growth. Furthermore, in microfluidic system, these extracts and their active constituents, characterized using High-Performance Liquid Chromatography (HPLC), showed a reduction in bacterial adhesion and biofilm formation.

Overall, these results highlight the promising potential of natural polyphenols, especially green tea and propolis, as alternative agents for preventing and treating biofilm-related infections.

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B50 Microbial Life in Extreme Environments: Insights from Bagno dell'Acqua Lake (Pantelleria, Italy)

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Lake Bagno dell'Acqua, located on the volcanic island of Pantelleria (Italy), is a remarkable hydrothermal environment characterized by high salinity, alkaline pH, and active geochemical gradients. The lake hosts a hydrothermal spring at about 55°C and slightly acidic which bring acidic and alkaline zones in close proximity and providing a unique natural laboratory that mimics early Earth and contemporary extreme environments. Notably, it harbors living microbialites potentially driven by microbially induced carbonate and siliceous precipitation [1-4].

Microbial community analysis reveals strong spatial heterogeneity and the presence of "boundary species" capable of inhabiting chemically distinct microhabitats, suggesting their role as ecological connectors. Special attention is given to microbial strategies for pH adaptation, osmotic stress resistance, and biomineralization processes, including carbonate precipitation.

Investigating microbial communities thriving across steep geochemical gradients enhances our

understanding of life's resilience to environmental stress, including adaptation to extreme pH and salinity. These systems not only reveal how microorganisms maintain functional stability and leave biosignatures under fluctuating conditions, but also offer valuable applications for industrial microbiology—such as bioremediation in harsh environments, extremozymes for biocatalysis, biosynthesis of carbonate biomaterials, and alternative fermentation technologies.

This study emphasizes the dual relevance of this Lake: as a model for reconstructing ancient biospheres and as a reservoir of microbial functions with potential applications in sustainable biotechnology and environmental innovation.

^[1] M. Cangemi et al., *Chem.Geol.* 2010, 276, 318–330

^[2] M. Cangemi et al., *Appl.Geochem.* 2016, 67, 168–176

^[3] C. Mazzoni et al *Front.Microbiol.* 2024, 15, 1391968

^[4] M. Ingrassia, et al., *Minerals* 2024, 14, 1013

B51

Environmental diversity, distribution, and evolution of microbial metal ion transport systems in global hydrothermal systems

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Transition metals are crucial for microbial metabolism, serving as catalytic enzyme cofactors or playing structural roles. Trace metal ubiquity in physiological processes demands their uptake from the environment. However, metal bioavailability under different redox states, along with potential toxicity, selected for tight homeostatic regulation. Microorganisms evolved several metal transport systems to cope with environmental trace metal concentrations, which have fluctuated during our planet history and potentially shaped the emergence and evolution of metal transporters. This study describes the diversity and distribution of microbial metal transport systems across several geothermal environments, where microbial metabolism is tightly linked to the elements supplied by water-rock interactions, providing an excellent model to investigate the diversity of microbial metal transport systems.

To this end, we performed shotgun metagenomics on samples collected from over 300 thermal features worldwide, and functionally annotated sequencing reads with a manually curated

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database of metal transport gene sequences. Metagenomics data were coupled to high-resolution geochemical analyses.

Our results suggest that the geochemical regime and trace metal abundance influence microbial metal transporters diversity and abundance. Specifically, transporter presence and abundance show an inverse correlation with environmental metal abundances. Metal uptake systems decrease with metal abundance, while efflux systems increase. These patterns suggest a dynamic regulatory mechanism, where microorganisms may adapt metal uptake strategies to fluctuating metal concentrations, offering novel insights into the evolution of metal transport systems. Such findings could have broad implications for understanding microbial evolution in extreme environments and the role of metal availability in the regulation of microbial diversity.

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Active layer microorganisms' contribution to carbon dioxide production in the warming Arctic

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Arctic regions are among the fastest warming areas of the planet. Increasing temperatures over the last five decades have deepened the thawing of the upper-most layer of permafrost, which contains significant amounts of organic carbon and nutrients. The progressive deepening of the seasonal thaw releases carbon and bioavailable nutrients used by active microorganisms, which in turn produce greenhouse gases, potentially onsetting a positive feedback on global warming. Despite their importance in controlling organic matter degradation and greenhouse gas production, data on microbial activity and dynamics in High Arctic soils in response to thaw are limited.

To investigate how nutrient availability affects microbial community structure and function, we performed a microcosm experiment using permafrost samples collected in Svalbard in June 2022. Microcosms were supplemented with iron and SL11 to simulate nutrient inputs, while a series of microcosms was used as a control. Over a 125-day incubation, we monitored geochemistry and microbial community shifts using gas sampling, 16S rRNA gene sequencing and metagenomics. Nutrient amendments led to shifts in community composition, suggesting nutrient mobilization during thaw may act as a strong environmental filter, selecting for specific functional groups and potentially altering biogeochemical cycles and greenhouse gas emissions. Carbon dioxide was produced in all three conditions, and isotopic compositions measured as $\delta^{13}\text{C-CO}_2$ supported a detrital origin of the carbon, eventually matching our field measurements. This study provides experimental evidence for microbial feedback associated with permafrost thaw and highlights the need for a better understanding of Arctic soil responses to climate change.

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MeStudio2 - A SnakeMake framework for epigenetics and multi-omics data integrationI. Passeri¹, C. Fagorzi¹, S. Pety², A. Mengoni¹¹Università degli Studi di Firenze, Sesto Fiorentino, Italy²INRAE, Jouy-en-Josas, France

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In prokaryotes, DNA methylation has been found implicated in several mechanisms such as DNA repair, gene expression, cell cycle progression and self-DNA recognition^[1].

Genomic DNA methylation patterns can vary between strains, affecting phenotypic variation and gene transfer: novel information gained in recent years are delving into the roles of transcriptional regulation and the formation of phenotypic variants^[2,3].

Interestingly, in 2022, Zhang et al. identified novel DNA methylation-regulated differentially expressed genes (MeDEGs) in RA integrating DNA methylation analysis and RNA-seq data. Starting from GSEA analysis, they've been able to find methylation-regulated genes and compare them to differentially expressed genes (DEGs) through correlation analysis^[4].

Moreover, Hi-C experiments demonstrated how the spatial organization of genomes is based on their hierarchical compartmentalization in topological domains. There is growing evidence that bacterial genomes are organized into insulated domains similar to the Topologically Associating Domains (TADs)^[5].

It's increasingly clear how DNA regulation is a multi-factorial mechanism: consequently, it is becoming highly relevant for molecular studies in bacteria to investigate the extent of the "pan-epigenome" and evaluate its role in genome adaptation and plasticity.

To deepen the understanding of such epigenetic mechanisms, it's important to fathom how the distribution of epigenetic markers (methylation, in particular) along the genome plays a functional role in the molecular regulation of genes, their corresponding transcripts and the 3D conformation of the chromosome(s).

To reach this goal, MeStudio2 is developed starting from MeStudio^[6] as a snakemake pipeline which crosses differentially expressed genes (DEGs), differentially methylated regions (DMRs) and motifs (DMMs) to find differentially methylated genes (DMGs) through statistical models and analysis. All these data can be crossed with Hi-C data, understanding how the differential distribution of epigenetic markers is affecting 3D spatial organisation of the DNA and consequently its expression.

References

¹Sánchez-Romero MA, Olivenza DR, Gutiérrez G, Casadesús J. (2020) *Nucleic Acids Res.* 48(21):11857-11867

²Oliveira PH. (2021) *mSystems* 00747-21.

³Vasu K, Nagaraja V. (2013) *Microbiol Mol Biol Rev.* 77(1):53-72.

⁴Zhang, R., Chang, C., Jin, Y. et al. (2022) *J Transl Med* 20, 481.

⁵Crémazy, F.G., Rashid, F.Z.M., Haycocks, J.R., Lamberte, L.E., Grainger, D.C., Dame, R.T. (2018) *Methods in Molecular Biology*, vol 1837.

⁶Riccardi, C., Passeri, I., Cangioli, L., Fagorzi, C., Fondi, M., & Mengoni, A. (2023). *International Journal of Molecular Sciences*, 24(1), 159.

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Adaptive Laboratory Evolution (ALE) and Microbial Electrosynthesis (ME) for the Capture and Transformation of CO₂ into Multicarbon Organic CompoundsR.A. Nastro¹, V. Pasquale¹, C. Avignone-Rossa²¹Laboratory of Microbiology and Biochemistry, Department of Science and Technology, University of Naples, Parthenope, Italy²Systems Microbiology Group, Department of Microbial Sciences, University of Surrey, Guildford, UK

A few microbial species can utilize C1 substrates to synthesize metabolic intermediates or precursors for biosynthesis of molecules for biomass production, intracellular carbon storage, and fermentation products. However, the assimilation of CO₂ can be achieved in species able to utilize electrons supplied exogenously. In the past twenty years, a new biotechnological process (the electrofermentation) has raised the interest of the scientific community worldwide. Electrofermentation exploits the influence of Extracellular Redox Potential (ERP) on intracellular redox homeostasis, therefore affecting gene expression, enzyme synthesis, signal sensing and transduction, and then, metabolic profile (Humphreys et al., 2018). In this work, we present a research aimed at demonstrating the CO₂ capture and conversion in added-value compounds in *Clostridium saccharoperbutylacetonicum* and *Cupriavidus necator* through electrofermentation in Bioelectrochemical Systems (BESs). For the first time in BESs, we applied an Adaptive Laboratory Evolution (ALE) approach for the selection of strains with better phenotypes by long-term culture under a specific selection pressure or growth environment. We also investigated the metabolic profile and intracellular PHBs content in *C.necator*, and formate, acetate, and 3-hydroxybutyrate in *C.saccharoperbutylacetonicum* when used for several thousands of generations in Microbial Fuel Cells (MFCs). The obtained profile was compared with the original strain purchased at DSMZ, which had never grown in BESs. Our results showed that the CO₂ utilization rate and metabolites change under the applied redox potential, with significantly improved CO₂ capture in BESs than in traditional microbial cultures. Metabolic modelling will be used to study the shift in metabolism of the used strains.

References

Humphreys CM, Minton NP. Advances in metabolic engineering in the microbial production of fuels and chemicals from C1 gas. *Curr Opin Biotechnol.* 2018 Apr; 50:174-181. doi: 10.1016/j.copbio.2017.12.023. Epub 2018 Feb 3. PMID: 29414057.

B55

Optimization of Indole-3-Acetic Acid Production by the Halophilic Bacterium *Halomonas titanicae*

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Indole-3-acetic acid (IAA) is a key phytohormone involved in the regulation of plant growth and development. Several plant-associated bacteria are known to synthesize IAA, influencing root architecture and plant-microbe interactions. In fact, this microbial feature is considered a valuable trait in plant growth-promoting bacteria (PGPB). In this study, we investigated the effects of nutrients and environmental factors on the regulation of IAA production in the marine bacterium *Halomonas titanicae*. The IAA levels were quantified by Salkowski assay across different temperatures, NaCl concentrations, pH values, carbon sources and inorganic nutrients. Moreover, waste products and seawater were tested as a low-cost production strategy. The optimal temperature for both IAA production and growth, used in all subsequent analysis, was 28 °C. The NaCl exerted an opposite effect, stimulating the IAA synthesis and inhibiting the growth. The

maximum production was at 1.0 M salt ($51.0 \pm 0.50 \mu\text{g/mL}$) after 48 hours. Optimal biosynthesis occurred at pH 8-9, while $\text{pH} < 7$ or ≥ 11 suppressed it. Glucose and sucrose stimulated IAA production more than mannitol or starch, while ammonium or phosphate slightly decreased it. Notably, we successfully cultivated *H. titanicae* in natural seawater, an inexpensive and readily available medium, achieving promising IAA production. Finally, buffalo milk whey supplemented with tryptophan also sustained IAA synthesis. These properties reinforced the biotechnological potential of microorganism and its suitability for the valorization of agro-industrial waste. Overall, these findings suggest *H. titanicae* as a robust candidate for biofertilizer applications, particularly in saline or low-nutrient environments.

B56	Characterization of microbiome involved in metal bioleaching from red muds
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Red mud is a waste material generated from the Bayer process, which is used for alumina extraction from bauxite. It is primarily composed of iron, aluminium, silicon, and titanium oxides. From an environmental standpoint, red mud represents a significant concern due to its highly alkaline pH, the toxicity associated with the presence of heavy metals, and the risk of environmental contamination resulting from inadequate management of disposal sites. Bioleaching is a process that utilizes the microbial production of organic acids to solubilize metals and other elements from solid matrices. Specifically, this process can be applied to red mud to extract commercially valuable elements, while simultaneously reducing its environmental hazard.

In the present study, the applicability of bioleaching was tested without preliminary biomass enrichment or bioaugmentation of selected strains. The presence of a microbial community of red mud was preliminary assessed through the enumeration of culturable microorganisms capable of proliferating within a pH range of 10 to 3. To investigate the metabolic processes involved in bioleaching, batch experimental systems were set up using red mud as a source of indigenous microorganisms and supplemented with different carbon sources. Monitored parameters were pH, DO, redox potential, substrate concentrations, key metabolic products, and target metals for recovery. The succession of the bacterial and fungal communities throughout the batch experiments is currently under evaluation by high throughput sequencing of the ribosomal genes. This work has been developed within the framework of the project e.INS www.einsardinia.eu [1] Next Generation EU- PNRR-M4 C2 I1.5 CUP F53C22000430001.

B57	Organic and inorganic additives released by bioplastics influence environmental bacteria growth
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Bioplastics are bio-based and/or biodegradable materials representing a valid alternative to conventional plastics, nowadays. Bio-based plastics are made of renewable resources to which additives are added to improve properties and performance. Inorganic and organic additives are

used as plasticizers, stabilizers and lubricating agents, and can migrate from bioplastics and be released in the environment, analogously to what happens for conventional plastics. Their impact on aquatic and terrestrial ecosystems has been largely neglected so far.

The effect of organic and inorganic additives released by bioplastics on the growth of model and environmental bacteria was here explored. Objects made of different bio-based polymers (e.g. PLA, starch/PBAT) were selected, leachates and organic extracts were obtained and tested on bacteria growth, considering both model and environmental bacterial strains. In addition, the influence of UV-aging on starch/PBAT objects' leachate was considered.

Organic and inorganic additives that can be released from bioplastics displayed different effects depending on the type of polymer and the test bacteria. Overall, leachates of starch/PBAT bioplastics stimulated microbial growth and can even act as a carbon source in a minimal medium; UV-aging didn't modify leachates effects. Organic extracts tend to inhibit bacterial growth, especially of gram-positive bacteria.

Although bioplastics are claimed to be more sustainable than conventional plastics, a deeper evaluation of the effects of additives released on the environment is needed.

B58 Engineering *E. coli* for Efficient and Sustainable PET Microplastic valorization

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Microplastic pollution, particularly from polyethylene terephthalate (PET), poses a severe threat to marine ecosystems and human health due to unsustainable consumption and disposal practices. The ProPla (Proteins from Plastic) project addresses this global challenge by converting PET microplastics from wastewater into the valuable amino acid alanine through advanced protein engineering and systems biology approaches. We engineered a novel biosynthetic pathway that channels PET breakdown products into the central carbon metabolism of *E. coli* K12 MG1655, enabling alanine biosynthesis. The pathway was then successfully integrated using CRISPR/Cas9 technology. Employing a bottom-up validation strategy, we confirmed the formation of key intermediates and demonstrated complete pathway functionality through both in vitro and cell-based bioconversion experiments. Additionally, in silico analyses, including Flux Balance Analysis, were performed to optimize process parameters, predict optimal growth conditions, and identify gene targets to maximize alanine yield. Establishing a stable microbial cell factory for the sustainable production of alanine from waste PET not only provides a potential route for mitigating plastic pollution but also enables the biotechnological valorisation of waste into high-value compounds.

The ProPla project is funded by Fondazione Cariplo under the call "Circular Economy: promoting research for a sustainable future - 2022."

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Comprehensive insights into Antibiotic-Resistant *Enterococcus faecium* isolated from Ready-to-Eat FoodsG. Piccioni¹, A. Di Cesare², G. Mangiaterra¹, I. Vaz Moreira³, C. Manaia³, A. Petruzzelli⁴, B. Citterio¹¹Department of Biomolecular Sciences, Section of Biotechnology, University of Urbino Carlo Bo, Fano, Italy²IRSA-CNR, Molecular Ecology Group, Verbania, Italy³UCP, Universidade Catolica Portuguesa, Centro de Biotecnologia e Química Fina (CBQF), Porto, Portugal⁴IZS, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, section of Pesaro, Italy

Enterococcus faecium is a commensal of the human and animal intestinal tract, increasingly implicated in nosocomial infections. Its detection in ready-to-eat (RTE) foods raises public health concerns due to the potential spread of antibiotic resistance genes (ARGs) through the food chain.

This study investigated *E. faecium* strains isolated from artisanal RTE meat products (e.g., Ciauscolo, sausages, salami) from the Marche region, assessing their antimicrobial resistance profiles, clonal diversity, and potential for horizontal gene transfer through phenotypic, molecular, and genomic approaches.

A total of 36 strains were isolated and analyzed by pulsed-field gel electrophoresis, revealing 22 distinct clones. Eight strains displayed resistance to at least one antibiotic; notably, two were resistant to linezolid, a last-resort drug used against vancomycin-resistant enterococci. Molecular analyses detected ARGs such as *tetM*, *tetL*, *ermA*, *ermB*, and *optrA*. All eight resistant isolates were strong biofilm producers. One strain was multidrug-resistant (MDR) and able to transfer linezolid resistance via filter mating. *In vitro* gastroduodenal digestion was performed to evaluate the survival of these resistant strains, which also underwent whole genome sequencing.

Bioinformatic analyses of the resistome, virulome, and plasmidome revealed mobile ARGs often encoded by conjugative transposons and plasmids. Phylogenetic analysis showed high genetic heterogeneity, indicating diverse evolutionary origins.

These results suggest that RTE foods can serve as reservoirs of MDR resistant bacteria and vehicles for ARGs dissemination, underlining the importance of monitoring antimicrobial resistance in the food chain and implementing preventive strategies in line with the One Health approach.

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The role of microbial communities in supraglacial ecosystemsF. Pittino¹, A. Crosta^{2,3}, R. Ambrosini⁴, B. Valle^{5,6}, L. Varchetta⁴, S. Mensa⁷, F. Ficaretola⁴, M. Gobbi⁷, V. Lencioni⁷, M. Caccianiga⁸, F. Paoli⁷, F. Dory¹, B. Leoni¹, A. Franzetti¹¹Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy²Department of Ecology, University of Innsbruck, Innsbruck, Austria³Austrian Polar Research Institute, Vienna, Austria⁴Department of Environmental Science and Policy, University of Milano, Italy⁵Department of Life Sciences, Università degli Studi di Siena, Italy⁶National Biodiversity Future Center (NBFC), Palermo, Italy⁷Research & Museum Collections Office, Climate and Ecology Unit, MUSE-Science Museum, Trento, Italy⁸Department of Bioscience, University of Milano, Italy

Forni Glacier is one of the most extensively studied glaciers in the Alps. Recently, a multidisciplinary project was launched to characterize its supraglacial ecosystem. In this study, we describe the algal, bacterial, and fungal communities of this environment using amplicon sequencing (Illumina

platform). We analyzed samples from various habitats, including ice, cryoconite, supraglacial debris, moraine, and *bediere*. Distinct microbial profiles were observed across habitats. Overall, we identified 2,691 bacterial, 857 algal, and 453 fungal Amplicon Sequence Variants (ASVs). A large proportion of algal ASVs remained unclassified. All habitats shared a notable percentage of ASVs—11% for bacteria, 22% for algae, and 33% for fungi. *Bediere* showed the highest proportion of unique ASVs for bacterial and algal communities (20%), whereas fungal ASV uniqueness was similar across all habitats (1–3%).

Our findings suggest that much of the biodiversity of Forni Glacier's supraglacial environment remains undocumented, particularly for algal communities. The elevated bacterial and algal diversity in *bediere* may be linked to the broader surface area drained by supraglacial streams. In contrast, the more uniform distribution of fungal ASVs could be attributed to their efficient spore dispersal, which likely leads to more homogeneous dispersion across the glacier, while bacteria and algae may experience stronger habitat-specific selection.

B61	Environmental Dimensions of Antibiotic and Heavy Metal Resistance in Freshwater Sediments
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The environmental spread of antibiotic resistance genes (ARGs) is a growing threat to global public health. Freshwater sediments, particularly in anthropogenically impacted basins, can act as long-term reservoirs of ARGs and metal resistance genes (MRGs). Once introduced into the environment, these genes can persist, mobilize, and re-enter clinical settings via horizontal gene transfer, closing a critical cycle between ecosystems and human health. In this study, we investigated the occurrence and distribution of ARGs and MRGs in Lake Maggiore (Verbania, Italy), a subalpine lake with a documented history of industrial, agricultural, and urban discharge. Sediment samples, collected from both coastal and deep sites at Fondo Toce, Suna Canottieri, and Teatro Maggiore, have been analysed through a multidisciplinary approach to investigate the presence, distribution, and ecological drivers of ARGs and MRGs. Microbial community profiling revealed a dominance of Planctomycetes, Actinobacteria, Bacteroidetes, and Chloroflexi, phyla potentially implicated in the spread of antimicrobial resistance in environmental settings due to their genetic versatility and role in mediating gene transfer. Moreover, resistance genes have been identified by annotating metagenomic data with Hidden Markov models (HMMs) against curated databases. In parallel, a culture-based approach allowed the isolation of antibiotic-resistant bacterial strains currently under characterization to assess co-resistance to heavy metals. Our study demonstrates that an integrative approach is key to comprehensively understanding the ecological mechanisms underlying the persistence and spread of antimicrobial resistance.

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BIO2BIO: Design and Application of Biosurfactant-functionalized Biochar for Enhanced Bioremediation

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Biosurfactants are molecules of microbial synthesis able to reduce surface tension between water and hydrophobic contaminants.

Biochar, a stable, carbon-rich material, has a structure which can provide a habitat for microbial communities.

The synergistic effect of combining biochar and biosurfactants in soil bioremediation processes could be exploited to enhance the biodegradation of organic pollutants.

The BIO2BIO project aims to demonstrate the technical and economic feasibility of applying Biosurfactant-functionalized Biochar for the bioremediation of hydrocarbon-polluted soils. This approach not only seeks to remediate polluted environments but also to valorise agricultural wastes used to produce the amendments.

Different feedstocks, pyrolysis and fermentation conditions were investigated to respectively obtain biochar and biosurfactants. The proper conditions for biochar functionalisation have been screened at a laboratory scale. After these preparatory investigations, the optimal feedstocks and production conditions have been selected to upgrade biochar and biosurfactants' manufacturing at an industrial scale to be applied for ex-situ bioremediation test.

A set of mesocosms have been implemented to test the addition of different concentrations of biochar and biosurfactants to the contaminated soil to evaluate the optimal doses for the novel product's formulation. Based on the results obtained from this test, Biosurfactant modified Biochar will be applied at the field-scale.

The process parameters will be monitored for the whole duration of the field-scale experimentation with the implementation of a specifically engineered sensor system able to detect deviations of the parameters from the optimal value, and to act to restore the ideal conditions for microbial catabolism.

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Sustainable lipopeptides production by *Bacillus subtilis* AC7 using rice husk as a renewable substrate after enzymatic treatmentA.C. Sansotera¹, C. Ceresa¹, C.F. Trejo², S. Morel¹, S. Aprile¹, G. Allegrone¹, M. Rinaldi¹, L. Fracchia¹¹Department of Pharmaceutical Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy²ROELMI HPC srl, Origgio, Varese, Italy

Biosurfactants are promising alternatives to synthetic surfactants due to their multifunctional properties and biodegradability; however, large-scale production is hindered by the high cost of raw materials and processing. In this context, the bioconversion of agro-industrial residues, rich in lignin, cellulose, and hemicellulose, offers a sustainable and low-cost carbon source for microbial fermentation. In this study, we investigated the potential of the endophytic strain *Bacillus subtilis* AC7 to produce lipopeptide biosurfactants using rice husk (RH) as a renewable substrate. RH was pretreated with a recombinant laccase, followed by starch hydrolysis with α -amylase and glucoamylase to increase nutrient availability. Laccase concentration was optimized in shake flasks, and the process scaled up to a 1-L bioreactor for improved control of fermentation parameters. For each condition, bacterial growth, supernatant surface tension, and biosurfactant yield were evaluated. In flask experiments, all treated RH substrates significantly increased microbial growth compared to untreated RH. The combination of 80–100 U/g laccase and starch hydrolysis resulted in the greatest reduction of surface tension. The highest biosurfactant yield was obtained after 72 h in medium containing 2.5% RH treated with 100 U/g laccase, combined with starch hydrolysis and supplemented with 3 g/L NaNO₃. LC-MS analysis of crude extracts confirmed the production of lipopeptide surfactin, with yields of 58.6 mg/L (untreated RH), 153.7 mg/L (hydrolyzed RH), and 237.5 mg/L (laccase pretreatment). Future work will assess the lipopeptide properties, including its antimicrobial activity, emulsification capacity, and oil-dispersing performance, to evaluate its potential for application in biomedical, cosmetics, and other industrial sectors.

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Development of an *Escherichia coli* platform to exploit higher L-threonine production for high value-added chemicalsT. Sassi¹, L.G. Brambilla¹, G. Rebuzzini², B. Teusink³, M. Vanoni¹¹University of Milano-Bicocca, Italy²AMSA SpA, Milan, Italy³Vrije Universiteit, Amsterdam, The Netherland

The development of novel and sustainable bioproduction routes remains one of the driving forces behind a greener chemical industry. The growing demand for amino acids, driven by the pharmaceutical and nutritional supplements industries, makes these chemicals an interesting field for developing novel bio-based production strategies. Using genome-wide metabolic models and Flux Balance Analysis, we are devising an *Escherichia coli* strain to produce L-threonine through previously undescribed routes. *In silico* simulations and preliminary *in vivo* experiments show that diverting carbon flux away from serine metabolism may be beneficial to enhance threonine production. This system may be further explored not only for the production of threonine itself, but also for developing an *Escherichia coli* platform able to produce high value-added products which require threonine as a starting substrate.

B65

A novel 1,2-dichloroethane degrading *Ancylobacter* in a consortium for enhanced aerobic bioremediationL. Scirè Calabrisotto¹, E.M. Petta², A. Vassallo³, G. Carpani⁴, M. Tagliavia⁵, V. Catania⁶, P. Quatrini⁶¹Department of Engineering, University of Palermo, Italy²Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy³School of Biosciences and Veterinary Medicine, University of Camerino, Italy⁴Environmental and Biological Laboratories, Eni S.p.A., Milano, Italy⁵Institute for Biomedical Research and Innovation – National Research Council (IRIB-CNR), Palermo, Italy⁶Department of Earth and Marine Sciences, University of Palermo, Italy

1,2-dichloroethane (1,2-DCA) is a toxic chlorinated hydrocarbon frequently found as soil and groundwater contaminant. Under aerobic conditions it can be biodegraded by few known specialized bacteria, mainly affiliated to Xanthobacteriaceae, in the genera *Xanthobacter* and *Ancylobacter*, isolated from different geographic locations. The aim of this study was to isolate novel 1,2-DCA degrading bacteria to be exploited in enhanced aerobic bioremediation strategies. A dechlorinating enrichment culture obtained from 1,2-DCA contaminated groundwater was streaked on solid mineral medium amended with 1,2-DCA as sole carbon source for bacterial isolation. Using IonTorrent 16S rRNA gene sequencing we discovered that the apparently single colonies were indeed similar stable consortia dominated by *Ancylobacter*, with *Ochrobactrum* and two other less represented genera. The consortia degrade 1000 ppm 1,2-DCA in three days. The *dhIA* gene encoding for the key enzyme of 1,2-DCA dechlorination, identical to that of all other known hydrolytic 1,2-DCA-degraders, usually plasmid-borne, was detected and located on *Ancylobacter* chromosome by Whole Genome Sequencing, as well as the other genes involved in 1,2-DCA hydrolytic dechlorination. Transposase genes flanking catabolic genes confirm that horizontal gene transfer can spread the 1,2-DCA degrading phenotype. We propose that *Ancylobacter* is the main degrader after comparing *dhIA* and *Ancylobacter* 16S rRNA genes abundance over time by qPCR; the role of the other members could be supporting the degradation by removing toxic intermediates. *Ancylobacter* whole genome can help gaining insight into 1,2-DCA dechlorination genes organization. The high degradation efficiency at such a high 1,2-DCA concentration makes consortia exploitable for bioaugmentation applications.

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Characterization of a Multi-Tolerant *Talaromyces funiculosus* from an Acidic Extreme Environment and Its Application in Soybean Growth PromotionL. Staiano¹, S. Castaldi¹, M. Masi², A. Cimmino², R. Isticato^{1*}¹Department of Biology, University of Naples Federico II, Complesso Universitario Monte S. Angelo, Italy²Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte S. Angelo, Italy

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Extremophilic fungi represent an unexploited source of plant growth-promoting agents, particularly in the context of increasing environmental stresses. In this study, we report the isolation and characterisation of a *Talaromyces funiculosus* strain isolated from “Piccolo Inferno”, an extremely acidic environment (pH 2.13) in Campania, Italy. Molecular identification was achieved through 18S rRNA and ITS region sequencing. Given previous reports of its plant growth-promoting potential, we evaluated this isolate’s ability to tolerate abiotic stresses and produce beneficial metabolites. The fungus exhibited remarkable resistance to high salinity (NaCl) and heavy metal stress, particularly copper (CuSO₄). Screening for hydrolytic enzyme production revealed its

ability to secrete multiple enzymes involved in nutrient cycling, in particular it is able to produce a large amount of cellulases without induction. Ethyl acetate extraction of the culture supernatant, followed by GC-MS/MS analysis, revealed a complex profile of bioactive secondary metabolites that could play a role in stress mitigation and growth promotion. To evaluate its beneficial effects, we conducted *in vitro* (germination tests) and pot experiments (plant growth promotion tests) using soybean (*Glycine max* L.), a crop for which *T. funiculosus* has previously been reported to act as a growth-promoting agent. Under saline and copper stress conditions, treated seeds and plants exhibited significantly improved growth parameters compared to controls, indicating the strain's potential to alleviate abiotic stress and enhance crop resilience. Our findings suggest that this extremophilic strain of *T. funiculosus* is a promising candidate for developing sustainable biofertilizers, particularly for use in degraded or contaminated soils.

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Yeast-insect associations: getting deeper into *Saccharomyces cerevisiae*-social wasp interactions and finding new natural connections

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Yeasts are widespread and form important ecological relationships, yet their ecology remains poorly understood. Recent studies have highlighted complex interactions among yeast populations, their vectors, and the environment. Our previous research revealed a strong association between *Saccharomyces cerevisiae* and social wasps, which supports yeast persistence, dispersal, and sexual reproduction in natural environments. Building on this, we examined how environmental and vector-related factors shape yeast ecology and evolution. Field surveys in Piedmont vineyards—chosen for varying environmental conditions and human influence—showed that yeast abundance and diversity were higher near woods. Notably, *S. cerevisiae* was found only in vineyards close to wooded areas and vectored exclusively by social wasps (*Polistes* spp. and *Vespa crabro*). In contrast, *Starmerella bacillaris* showed no such specificity, and genomic analysis revealed unique traits compared to strains from other environments. The specificity of the *S. cerevisiae*-wasp relationship led us to investigate the physiological and environmental conditions supporting it. Molecular and chemical analyses indicated that factors like pH, viscosity, and amino acid presence in the wasp gut promote yeast survival, as also confirmed by yeast molecular profiles (transcription and deletion analyses). Understanding yeast ecology demands a multidisciplinary approach. Studying yeast-insect associations offers valuable insights into the ecological and evolutionary dynamics of natural yeast populations.

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Long-term dynamics of microbial communities in bioelectrochemical systems applied for the remediation of a chronically contaminated marine site in Chile

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Microbial electrochemical technologies (METs) may be a suitable strategy for the bioremediation of petroleum hydrocarbon-polluted sediments, as electrodes provide a sustainable and inexhaustible source of electron acceptors. This study investigated the long-term performance of bioelectrochemical systems (BESs) for the anaerobic bioremediation of chronically petroleum hydrocarbon-contaminated sediment from a brownfield in Viña del Mar, Valparaíso, Chile. Three BES-based approaches were implemented at a laboratory scale, including a microbial fuel cell (Closed Circuit), a microbial electrochemical cell (PoI-MERC), and a microbial electrochemical snorkel (Snorkel). These systems were run in parallel with control systems (Open Circuit, Natural Attenuation). Additionally, compost was incorporated into the sediment matrix to enhance the remediation process. Our investigation focused on the remediation processes, biogeochemical dynamics, and the succession of microbial communities over a two-year incubation period. Monitoring analyses of the most recent experimental sampling are currently ongoing. Total petroleum hydrocarbon concentrations were monitored through GC-FID analyses to assess biodegradation rates. Biogeochemical dynamics were investigated by analysing total iron, iron (II), sulphate, nitrate, and ammonium. The composition of microbial communities was analysed through 16S rRNA gene sequencing of the metagenomic DNA extracted from the sediment and electrode samples. Sequencing data will also be used to predict the hydrocarbon catabolic potential (by using the CANT-HYD database) and the metabolic potential (by using the FAPROTAX database) of microbial communities. All these results will be useful to better understand the main terminal electron acceptors involved in enhancing the bioremediation process, as well as the roles of electrochemically active bacteria and hydrocarbon-degrading bacteria.

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Influence of Agronomic Management and Wheat Cultivars on GHG emissions and Metabolic Fingerprinting of Soil Microbial Communities

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Wheat (*Triticum aestivum* L.) is one of the most cultivated cereal crops in the world. The interactive effects of wheat genotype and management practices on soil GHG emissions and metabolic diversity are poorly explored and require site-specific studies. This study aims to evaluate the metabolic profiles of microbial communities and GHG emissions in agricultural soils cultivated with different wheat cultivars, using conventional and organic management. Soil was collected at various stages of wheat development in two distinct locations in Italy. Preliminary results based on community-level physiological profiling (CLPP) using Biolog™ system indicate that samples from organically managed systems exhibited higher average well colour development (AWCD) than those from conventional systems, suggesting greater metabolic activity. When cultivars are considered separately, Bologna displays the highest and Nogal the intermediate AWCD, while Sieve records the lowest AWCD, yet still exhibits higher values under organic than conventional management. Measurements of GHG emissions using portable gas analyzers showed that in Bologna cultivar at flowering, CH₄ and CO₂ fluxes were lower in organic than conventional management, with no significant differences at later stages, while N₂O emissions were similar in both management systems. CO₂ detection revealed no significant differences between Sieve and Nogal cultivars or cultivation methods at the tillering phase, whereas CH₄ and N₂O emissions were higher in Sieve than in Nogal cultivars, regardless of the management system. These two last cultivars did not show different emissions between the two management practices. Overall, integrating CLPP and GHG data indicates clear genotype-by-management interaction within the soil system.

This study was carried out as part of the National Center for Technology in Agriculture (AGRI-TECH) funded by the National Recovery and Resilience Plan (PNRR) of the European Union – MISSION 4 COMPONENT 2, INVESTMENT 1.4 – D.D. 1032 17/06/2022, CN00000022.

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B70 On the diversity and distribution of nickel metalloenzymes in extreme environmentsE. Taccaliti^{1*}, G. Climent Gargallo^{1*}, A. Cordone¹, D. Giovannelli^{1,2,3,4,5}¹Department of Biology, University of Naples Federico II, Italy²Institute of Marine Biological Resources and Biotechnologies, National Research Council, Ancona, Italy³Earth-Life Science Institute, Tokyo Institute for Technology, Tokyo, Japan⁴Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA⁵Department of Marine and Coastal Science, Rutgers University, New Brunswick, NJ, USA

Life harvests the energy to sustain itself from redox chemical reactions. Oxidoreductases, the enzymes catalysing these reactions, exploit transition metals in their active sites, such as Fe, Co, Ni, Mo, W, V, and Cu, due to their availability and occurrence in multiple oxidation states. Among all, nickel plays an essential role in hydrogen and carbon metabolism, being fundamental for NiFe hydrogenases and key carbon fixation enzymes of the Wood-Ljungdahl pathway, as well as participating in methanogenesis. Despite the biological relevance of this metal in the evolution of microbial metabolism, the diversity and distribution of nickel metalloenzymes have been poorly investigated. The present study aims to describe these proteins in metagenomes collected from hundreds of extreme environmental features across the world. To this end, we combined shotgun metagenomic sequencing and geochemical analyses to disentangle the interactions between the availability of nickel and microbial metabolic diversity. Sequencing outputs were functionally annotated using Hidden Markov Models based on a custom database of nickel metalloenzymes to correlate these genes to the environmental concentrations of this metal. Our results show how these oxidoreductase abundances are associated with nickel concentration, together with the physico-chemical and geochemical properties of the respective sampled environments.

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B71 Biological characterization and genetic manipulation of the enediyne-encoding gene cluster of *Nonomuraea gerenzanensis* ATCC 39727A. Talà¹, M. Calcagnile¹, F. Damiano², S.C. Resta², S.M. Tredici², S. Davideb^{2,3}, O. Yushchuk⁴, F. Berini⁵, F. Marinelli⁵, P. Alifano²¹Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy²Department of Experimental Medicine, University of Salento, Lecce, Italy³Department of Medical Biotechnologies, University of Siena, Italy⁴Department of Genetics and Biotechnology, Ivan Franko National University of Lviv, Ukraine⁵Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

Nonomuraea gerenzanensis ATCC 39727 is an industrially relevant microorganism, used for the production of the lipoglycopeptide A40926, which is the precursor of dalbavancin. Genome mining with antiSMASH platform revealed the presence of several clusters coding for many other secondary metabolites, including polyketides, non-ribosomally synthesized peptides, lantipeptides and terpenes. In this study, we focused on cluster 12, which exhibited considerable homology with the biosynthetic gene cluster coding for the enediyne C-1027 of *Streptomyces globisporus*. Eneidyne compounds are considered nowadays very promising molecules in anticancer therapy due to their ability to produce single stranded or double-stranded DNA breakage. Preliminary RNAseq data indicated that cluster 12 is expressed in *N. gerenzanensis*, grown in a basal medium. Here, we provide new evidence for enediyne production in *N. gerenzanensis*, using an *in vitro* DNA nicking assay, performed on exhausted broths. In our assays, DNA nicking activity

was detectable only in a restricted interval of growth, ranging from 48 to 96h. This result could be related to the expression levels of a noncovalently enediyne-bound protein, called apoprotein, that stabilizes and prevents a spontaneous degradation of the labile enediyne. Interestingly, we found that overexpressed and purified apoprotein was able to anticipate and enhance the DNA nicking activity of exhausted broths. Moreover, inactivation and overexpression of a putative enediyne synthase in *N. gerenzanensis* caused loss or early enediyne production, respectively. Overall, our study leads the way for further investigations aimed at the purification of the enediyne molecule and at the characterization of its structure and biological activity.

B72 Antifungal effects of ozonated olive oil

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The increasing prevalence of resistance mechanisms and adverse effects linked to conventional antifungal agents underscores the necessity for alternative therapeutic options. This research investigates the antifungal efficacy of ozonated extra-virgin olive oil (EOO) against *Candida albicans*, aiming to develop environmentally friendly and highly potent treatments derived from natural sources. Antifungal activity was assessed through cell viability and biofilm formation assays, employing Crystal Violet and Sytox Green staining techniques. Findings indicated that EOO decreased *C. albicans* viability in a concentration-dependent manner, with over 90% of cells eliminated at a 3% (v/v) dose. Transmission Electron Microscopy (TEM) analysis demonstrated structural disruptions in the cell wall, while reactive oxygen species (ROS) levels increased by approximately 60% within 10 minutes of exposure compared to untreated controls. Furthermore, the expression levels of autophagy-associated genes atg-7 and atg-13 were upregulated by 2- and 3.5-fold, respectively, after 15 minutes, indicating activation of stress-induced cell death pathways. EOO also markedly suppressed hyphal development and biofilm formation, thereby diminishing the pathogenic potential of *C. albicans* while maintaining biocompatibility. Similar antifungal effects were observed against *Candida glabrata*. In summary, ozonated olive oil exhibits significant antifungal activity by decreasing cell viability, impeding hyphal and biofilm formation, and inducing oxidative stress and autophagic responses. These results support the potential of EOO as a natural, effective alternative for managing fungal infections.

B73 Microbial diversity and biotechnological potential of halophilic microorganisms from salt ponds of Trapani

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Keywords: Halophiles, Saltern ponds, antioxidant assay, Carotenoids, Metabolomics

Hypersaline environments such as salt ponds represent ecological niches with unique physicochemical conditions—high salt concentration, intense solar radiation, and variable temperature—that strongly shape microbial communities^{1,2}. The Natural Reserve “Saline di Trapani e Paceco” (Sicily, Italy), covering ~1000 hectares and subjected to seasonal salt extraction cycles, offers a dynamic and underexplored model system for the study of extremophilic

microorganisms. In this study, we performed an integrated approach combining physico-chemical characterization of the saltern waters, culture-dependent and culture-independent (18S and 16S rRNA metabarcoding) methods to assess microbial diversity, with a focus on both eukaryotic microalgae and prokaryotes, including halophilic archaea. Several microorganism strains were isolated and cultivated under different salinity regimes. Pigment analysis and untargeted metabolomic profiling revealed high chemical diversity, including carotenoids (e.g., β -carotene) and osmoprotectants (ectoine, betaine), which were more abundant under hypersaline conditions. Extracts were tested for antioxidant activity (DPPH assay) showing promising biotechnological potential. Metabarcoding data confirmed a rich and stratified microbial community, dominated by halophilic and extremely halophilic taxa, including *Dunaliella* spp., *Dactylococcopsis salina* and *Haloarchaea*. This multidisciplinary investigation provides an overview of the Trapani salterns as a hotspot of extremophilic biodiversity and a source of novel bioactive compounds with potential applications in biotechnology, pharmaceuticals, and cosmeceuticals.

Reference

¹Cusenza, B.S., Scelfo, G., Licata, G., Capri, F.C., Vicari, F., Alduina, R. and Villanova, V. (2025), First Insights Into the Biological and Physical–Chemical Diversity of Various Salt Ponds of Trapani, Sicily. *Environmental Microbiology Reports*, 17: e70075. <https://doi.org/10.1111/1758-2229.70075>

²Villanova V., Galasso C., Fiorini F., Lima S., Brönstrup M., Sansone C., Brunet C., Brucato A., Scargiali F. Biological and chemical characterization of new isolated halophilic microorganisms from saltern ponds of Trapani, Sicily. *Algal Research*, Volume 54, 2021, 102192, ISSN 2211-9264, <https://doi.org/10.1016/j.algal.2021.102192>.

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B74 ESKAPEE Gram-negative bacteria escape environmental detection

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ESKAPEE bacterial pathogens (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*) represent a public health threat due to their increasing resistance to multiple classes of antibiotics. The hospital environment, including various surfaces and medical devices, is widely recognized as a critical reservoir for pathogen transmission, playing a key role in patient cross-infection. Conventional methods for assessing microbial contamination rely on culture-based techniques, which cannot detect bacterial cells that have entered the viable but non-culturable (VBNC) state. VBNC cells remain alive and potentially infectious but lose the ability to grow on standard microbiological media commonly used in environmental surveillance. The aim of this work is to investigate entrance into the VBNC state upon desiccation of ESKAPEE pathogens on surfaces mimicking those found in hospitals. To this purpose, bacteria were desiccated for one week on glass, plastic polymers, cotton, and titanium. After desiccation, a reduction in culturability was observed in all tested strains, which varied depending on the bacterial species and surface type. To determine whether the reduction in bacterial recovery was due to cell death or a VBNC transition, desiccated cells were incubated in a carbon-free resuscitation buffer at 37°C for 24 hours. Gram-positive ESKAPEE pathogens failed to recover their culturability upon resuscitation, whereas Gram-negative bacteria regained culturability, although to a different extent depending on the species and strain, indicating a transition to the VBNC state in response to desiccation

stress. By comparing our resuscitation-based approach with commercially available swabs and the standardized method for biocontamination control (EN 17141:2020), we demonstrated that traditional techniques fail to detect ESKAPEE Gram-negative bacteria in the VBNC state, highlighting the urgent need for improved detection methods targeting VBNC pathogens contaminating abiotic surfaces.

B75 **Genome-Driven Mechanisms and Molecular Insights into Polyethylene Degradation by *Rhodococcus opacus* R7**

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Polyethylene (PE) is the most abundant and persistent synthetic plastic, representing a major fraction of global plastic waste. Its physicochemical recalcitrance poses relevant environmental and biotechnological challenges. Microbial degradation is emerging as a promising approach, particularly through genomic and transcriptomic strategies.

Rhodococcus opacus R7 is capable of growing on untreated PE as the sole carbon and energy source. RNA-seq analysis has revealed key genes activated during this process, establishing R7 as a valuable model for plastic biodegradation studies.

In this study, the genome-driven molecular response of *R. opacus* R7 under different PE exposure conditions was assessed, including varying cultivation settings, PE concentrations (1% single-dose vs. 0.4% fed-batch), and the presence of potential inducing substrates. Notably, pre-cultivation on PE exerted a slowdown effect on R7, leading to altered growth in subsequent inocula and increased lipid accumulation over extended cultivation. In contrast, the 0.4% PE fed-batch condition elicited a broader and more dynamic transcriptional response, suggesting a finely regulated system responsive to substrate concentration. Beyond the differential expression of key genes encoding oxidoreductases, such as alkane monooxygenases, cytochrome P450 hydroxylases, and laccase-like multicopper oxidases (LMCO1, LMCO2, and LMCO3) detected by the transcriptomic approach, additional multicopper oxidases and P450 hydroxylases were up-regulated under specific PE degradation conditions.

Moreover, lignin-derived compounds (e.g., ferulic acid) and long-chain *n*-alkanes (e.g., C24) activated diverse LMCOs and oxidoreductases. These findings suggest that *R. opacus* R7 employs multiple coordinated strategies to initiate and sustain PE degradation and provide novel molecular targets for biotechnological applications in sustainable plastic waste valorization.

B76 **High density polyethylene (HDPE) biodegradation by *Cladosporium halotolerans***

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Polyethylene (PE) is high molecular weight synthetic polymer, very hydrophobic and hardly biodegradable. To increase polyethylene bio-degradability it is very important to find microorganisms that improve the PE hydrophilic level and/or reduce the length of its polymeric chain by oxidation. In this study, we isolated *Cladosporium halotolerans*, a fungal species, from the gastric system

of *Galleria mellonella* larvae. Here, we show that *C. halotolerans* grows in the presence of PE polymer, it is able to interact with plastic material through its hyphae. Formation of erosion, defects and holes observed in SEM micrographs suggest that this fungus adheres to the surface where the degradation process occurs, and carbonaceous compounds are released in the medium. The *C. halotolerans* supernatant protein pattern analysis revealed some differences associated with polyethylene presence, the most significant is the presence of FAD_binding_3 domain containing enzyme, a protein that binds FAD as prosthetic group used by various oxidoreductase enzymes probably involved in PE degradation.

Session C - Interactions between microbes/viruses and their hosts

[Continues from Oral session C. [Click here to view the abstracts from C1 to C12](#)]

C13

The Endophyte *Klebsiella pasteurii* BDA134-6 triggered key physiological and biochemical responses in wheat under water deficit

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Climate change poses a serious threat to global food security by intensifying abiotic stresses, such as drought, high salinity, and extreme temperatures, all of which severely reduce plant productivity^[1]. Among them, drought stress has the most profound impact on plant physiology, morphology, and metabolism, impairing photosynthesis, nutrient uptake, and cellular integrity through the excessive accumulation of reactive oxygen species^[2]. The use of Plant Growth-Promoting Bacteria (PGPB), able to stimulate growth and stress tolerance through direct (e.g., phytohormone production, nitrogen fixation, phosphate solubilization) and indirect mechanisms (e.g., pathogen antagonism)^[3], have emerged as promising strategy to enhance plant resilience in a sustainable and cost-effective manner. Among PGPB, endophytes, that inhabit the interior plant tissues without causing damage, are particularly effective in mitigating drought stress through the improvement of physiological and biochemical plant responses. This study evaluated the potential of *Klebsiella pasteurii* BDA134-6, an endophyte isolated from *Oryza glaberrima*, to colonize *Triticum durum* (cv. Primadur) and enhance its drought tolerance. Experiments under laboratory, phenotyping platform, and greenhouse conditions revealed that inoculated wheat plants maintained significant acetylene reduction activity under drought conditions. Furthermore, they exhibited reduced H₂O₂ accumulation, higher levels of proline and ascorbic acid, increased antioxidant enzyme activity, limited lipid peroxidation, and delayed leaf senescence compared to uninoculated control plants. Our findings demonstrate that the inoculation of wheat plants with *K. pasteurii* BDA134-6 effectively enhances its drought resilience, highlighting the potential of PGPB-based approaches for sustainable agriculture.

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References

- ^[1] Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, 529(7584), 84–87
- ^[2] Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, 29(1), 185–212
- ^[3] Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., & SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*, 184, 13–24

C14**Flagellin-driven zinc redistribution as a plant immune strategy against *Salmonella* Typhimurium**T.A. Mandava^{1,2}, E. Michetti¹, M.L. Astolfi³, L. Camoni¹, A. Battistoni¹, S. Visconti¹, S. Ammendola¹¹Department of Biology, Tor Vergata University of Rome, Italy²PhD Program in Evolutionary Biology and Ecology, Tor Vergata University of Rome, Italy³Department of Chemistry, Sapienza University of Rome, Italy

Salmonella enterica serovar Typhimurium (STM) can colonize the apoplast of plants, including edible species, making them potential reservoirs and sources of human infection. We previously showed that STM exploits its ability to export zinc via the P_{1B}-type ATPase ZntA, induced by zinc excess, to colonize *Arabidopsis thaliana* Col-0 shoots. A mutant plant line with reduced Zn translocation from roots to shoots is more susceptible to STM colonization, suggesting that plants may use Zn intoxication as a defense strategy. Here, we show that upon STM colonization, *A. thaliana* alters the expression of zinc transporters, promoting long-distance Zn movement while limiting its storage in vacuoles. Notably, this response depends on the recognition of bacterial flagellin by the FLS2 receptor, which activates pattern-triggered immunity (PTI). Disruption of this interaction, either by using a flagellin-deficient STM (Δ fla) strain or an *A. thaliana* *fls2* mutant, leads to reduced expression of *zntA* in shoot-colonizing STM. This is further supported by luminescent Zn biosensor assays, which show that the *fls2* mutant does not increase bioavailable Zn levels upon STM colonization. Additionally, gene expression analysis of the *fls2* mutant reveals a downregulation of root-to-shoot Zn translocation compared to Col-0. Overall, our findings suggest that flagellin perception triggers a Zn-mediated defense mechanism that contributes to the plant immune response against bacterial invaders. These results highlight the role of Zn homeostasis in the interaction between plants and pathogens.

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C15

Fecal microbiome in Anorexia Nervosa: shotgun genomics sequencing reveals major differences with controls and longitudinal effects of inpatient treatment

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Anorexia Nervosa (AN) is a severe psychiatric disorder marked by extreme dietary restriction, significant weight loss, and psychological and physiological dysfunction. It primarily affects adolescent and young adult females and has one of the highest mortality rates among psychiatric conditions. Growing evidence implicates gut dysbiosis in AN pathophysiology, yet shotgun metagenomics remains underused, particularly in longitudinal contexts.

This study aimed to: i) Characterize gut microbiome composition and function in individuals with AN versus age-matched healthy controls; ii) Investigate longitudinal microbiome changes during and after AN treatment; iii) Identify microbial taxa (bacteria, archaea, viruses, fungi), gene families, and metabolic pathways associated with clinical outcomes, such as BMI and treatment duration. We analyzed the fecal microbiome of 151 AN patients at treatment admission and 74 age-matched controls from three European centers using shotgun metagenomics. A subset of 53 adolescent patients was sampled longitudinally at admission, discharge, and one year post-admission. Microbiome composition, functional potential, and metabolic fluxes were assessed in relation to clinical variables.

At admission, microbiome composition and function were significantly influenced by both geographic origin and health status. These features correlated with key clinical indicators. Longitudinal data showed partial microbiome restoration with weight gain, but significant differences persisted one year post-treatment. Several taxa and gene families were linked to treatment duration and long-term BMI, indicating their potential as biomarkers or therapeutic targets.

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C16

Investigating the metabolic-microbiome-immunological axis and exposome in a rural-to-urban transition in Sub-Saharan Africa

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Chronic consumption of ultra-processed food coupled with sedentary behavior causes immunometabolic alterations that may enhance systemic low-grade inflammation and increase non-communicable diseases globally. This lifestyle also affect the human gut microbiome composition by promoting the growth of harmful species while increasing the risk of intestinal dysbioses. Sub-Saharan Africa's rapid urbanization provides a natural experiment: rural communities preserve traditional diets and activity patterns, while urban dwellers adopt Westernized behaviors.

Objective: To assess how different exposomes affect the metabolic-microbiome-immunological axis in populations undergoing rural-to-urban transition in Burkina Faso.

Methods: We enrolled urban and rural family members in Burkina Faso, collecting blood, plasma, and stool samples alongside detailed diet and lifestyle questionnaires. Blood samples underwent gene expression profiling; plasma was analyzed via untargeted metabolomics; stool microbial DNA was subjected to shotgun metagenomics. Integrated multi-omics analyses, including Multi-Omics Factor Analysis (MOFA), were applied to identify exposome-related signatures.

Findings: Significant differences between members of families coming from different areas along the urban-to-rural transect were found. The assumption of fibers, vegetable proteins, and carbohydrates correlated with specific gene expression patterns, metabolite abundance, and gut microbiome structure. All results were also confirmed by the Multi-Omics Factor Analysis (MOFA) highlighting features associated with the different exposomes.

Conclusion: Westernized dietary and lifestyle transitions in Sub-Saharan Africa lead to measurable alterations in host immunity, metabolism, and microbiome architecture. Fiber consumption coupled with vegetable intake may bolster immune health and preserve the structure of gut microbiome.

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C17	Granuloma Like Structure (GLS), a model to evaluate drug efficacy and to characterize virulence in <i>Mycobacterium abscessus</i>
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Mycobacterium abscessus (*Mab*), a nontuberculous mycobacterium, primarily affects cystic fibrosis individuals and presents smooth (S) and rough (R) morphotypes. Like *Mycobacterium tuberculosis*, *Mab* can induce granuloma formation—structured immune cell aggregates aimed at containing infection. These granulomas create a challenging microenvironment with limited nutrients, oxygen, and drug penetration, which contributes to *Mab*'s persistence. The Granuloma-Like Structure (GLS) assay effectively mimics these conditions, making it a valuable model for both studying early host-pathogen interactions and evaluating potential drug treatments.

We optimized the GLS model induced by *Mab* infection in human monocytes observing that granulomas form after 24 hours of infection, visible at 4X magnification. Intracellular and extracellular bacterial growth was monitored by vital counts. We characterized this phase using different strains (clinical isolates, *Mab* S and R variants) for the infection, along with confocal microscopy for analysis. We tested the activity of drugs used in *Mab* therapy; interestingly, mefloquine, a repurposing compound, showed the best activity.

Furthermore, we are developing a tool to analyse microscopy images to extract the size distribution of GLSs, with the potential to distinguish between S and R variants based on differences in size, shape, or number.

These findings provide further insights into *Mab* infection, laying the basis to explain why it is becoming such a concerning pathogen.

This work is funded by Italian Cystic Fibrosis Research Foundation (FFC#9/2023) and EU funding NextGenerationEU-MUR PNRR Extended partnership on Emerging Infectious Diseases (Project PE00000007. INF-ACT).

C18	The TbD1/<i>mmpS6-mmpL6</i> deletion/inactivation is a crucial evolutionary event for the patho-adaptation of mycobacterial strains to human host
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Deletion of the TbD1/*mmpS6-mmpL6* locus is the genetic hallmark of the modern epidemic *Mycobacterium tuberculosis* (*Mtb*) lineages, etiologic agents of human tuberculosis. The loss of a functional TbD1/*mmpS6-mmpL6* region in *Mtb* strains results in enhanced virulence in animal models mirroring the human disease progression, and in increased resistance to oxidative stress. *Mycobacterium canettii* (*Mcan*), the phylogenetically closest relative of tuberculosis-causing mycobacterial strains, harbours an intact TbD1 locus. However, different *Mcan* strains (STB-A or STB-D) encode for MmpL6 variants, carrying the predicted MmpL6-inactivating T878M amino-

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acid substitution. In this study, the impact of TbD1 inactivation by T878M mutation on virulence and stress resistance was investigated. A panel of *Mcan* STB-A and/or *Mtb* TbD1-knock-out and TbD1-knock-in strains (expressing the wild-type or the STB-A/STB-D TbD1 variants) was constructed and characterised. The expression of the STB-A-TbD1 variant in the naturally TbD1-deleted *Mtb* had no impact on virulence in guinea-pig, neither on resistance to oxidative stress. Consistently, TbD1-deletion in STB-A did not affect the virulence in C3HeB/FeJ mice, neither the survival after exposure to hydrogen peroxide in vitro. Complementation of the STB-A TbD1-deletion mutant with the wild-type TbD1 (but not with the STB-A/STB-D TbD1 variants) confers virulence attenuation and increased sensitivity to oxidative stress, thus confirming the inactivating effect of the T878M mutation.

These data indicate that the MmpL6-inactivating mutations in STB-A/STB-D strains are a first attempt of patho-adaptation to the human host, and further confirm that the loss of a functional TbD1/*mmpS6-mmpL6* locus is a key event for the evolutionary success of globally-spread *Mtb* lineages.

C19	A hybrid biomaterial device incorporating antimicrobial peptides for oral Infection control
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Oral bacterial diseases are among the most widespread chronic conditions, impacting both quality of life and the progression of other systemic and neurodegenerative disorders. The growing issue of antibiotic resistance (AMR) highlights the urgent need for alternative treatments beyond conventional antibiotics. In this context, host defense peptides (HDPs) are emerging as promising bio-inspired antimicrobial agents due to their ability to target bacterial membranes and disrupt biofilms. A potential strategy to enhance the effectiveness of HDPs against oral pathogens involves their integration into 3D hybrid device. By combining these peptides with nanomaterials and incorporating them into polymer-based scaffolds, their stability and bioavailability can be improved. In this study, we employed a bioinformatics approach to identify novel antimicrobial peptides, focusing on SQQ30, a peptide derived from the human SOGA1 protein. We evaluated its antimicrobial activity, interaction with bacterial lipopolysaccharides (LPS), and its anti-inflammatory and antioxidant properties. We also evaluated the functionality of the hybrid 3D device PCL/CS (polycaprolactone/chitosan) incorporating SQQ30 against oral pathogens. The results confirmed the device's antimicrobial activity, further supporting its potential as a therapeutic approach. These findings strengthen the rationale for combining HDPs with biomaterials to combat oral infections and reduce reliance on conventional antibiotics.

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C20	Highly Potent Aromatic Isocyanides targeting Methicillin- and Vancomycin-Resistant <i>Staphylococcus aureus</i> infections
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Antimicrobial resistance is a source of great concern worldwide and a serious threat that requires the deployment of innovative molecules with new mechanisms of action. Multidrug-resistant *S. aureus* is one of the most common causes of hospital-acquired and post-surgical wound infections. A class of novel compounds containing an isocyanide functional group showed to be highly effective against methicillin/vancomycin-resistant *S. aureus* strains. Isocyanides 14 and 27 were identified as the most promising. They showed a bacteriostatic activity with MIC ranges from 0.06 to 0.5 μM and markedly prevented the formation of biofilms up to 97% starting from 0.125 μM . Unlike ciprofloxacin, the isocyanides did not induce resistance. When co-incubated with *C. elegans*, compounds were harmless to the worms and significantly increased survival of nematodes infected with *S. aureus* Mu50. Isocyanides were non-toxic to HaCaT and MRC5 mammalian cells up to 64 μM and stable on human epidermal skin S9 but showed low metabolic stability in mouse and human plasma, suggesting their applicability in the treatment of topical infections as soft drugs. Furthermore, the treatment with isocyanide 27 in a murine model of MRSA skin infection led to a significant reduction of bacterial counts, nearly matching the efficacy of fusidic acid, the current standard of care.

These results suggest that isocyanide can be used as a pharmacophore for the design and synthesis of lead candidates for preclinical and clinical investigations, which could be used in the future as new weapons against multidrug-resistant *S. aureus* wound infections.

This study is funded by PRIN2022.

C21	1,25-dihydroxyvitamin d3 affects sarscov2 replication in a 3d bronchial epithelial cell model
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Vitamin D3 exerts immunomodulatory, antiviral, and anti-inflammatory effects, and its deficiency has been associated with increased severity of COVID-19. However, the molecular mechanisms underlying its protective role remain unclear. Notably, Vitamin D3 is a known inducer of autophagy, suggesting that Vitamin D3-mediated autophagy may contribute to its antiviral activity during SARS-CoV-2 infection.

This study aimed to evaluate the antiviral effects of 1,25-dihydroxyvitamin D3 against SARS-CoV-2 *in vitro*.

The human bronchial epithelial cell line Calu-3 was differentiated into a pseudostratified epithelium using air-liquid interface (ALI) culture conditions. Differentiated ALI-Calu-3 cells were treated with 1,25-dihydroxyvitamin D3 at concentrations of 75, 100, and 130 pM. Autophagy

markers were assessed by western blot at 24, 48, and 72 hours. To evaluate antiviral activity, ALI-Calu-3 cells were infected with the SARS-CoV-2 BA.5 variant at a multiplicity of infection (MOI) of 0.1. 1,25-dihydroxyvitamin D3 was added at the indicated concentrations. Viral RNA in the apical supernatant and infectious titer (TCID50/ml) were measured at 24, 48, and 72 hours post-treatment.

1,25-dihydroxyvitamin D3 induced expression of the autophagy marker LC3B-II at 75 pM after 24 hours and p62 at all concentrations at 24 and 48 hours. Furthermore, 1,25-dihydroxyvitamin D3 at 75 pM significantly reduced viral RNA levels at 72 hours post-infection. A corresponding decrease in TCID50 was observed, indicating a reduction in the production of infectious viral particles.

These data provide the first evidence of the capacity of 1,25-dihydroxyvitamin D3 to limit SARS-CoV-2 replication in a differentiated bronchial epithelial model, likely through early autophagy activation.

C22

The Rcs phosphorelay system as a regulator of intracellular fitness in *Shigella flexneri*

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Intracellular survival, replication and colonisation by pathogens require constant monitoring of the environment. Two-component systems (TCSs), composed of a transmembrane histidine kinase (HK) sensor and a cytoplasmic response regulator (RR), are key bacterial tools for the transduction of environmental signals. The Rcs system is a non-orthodox phosphorelay TCS that senses outer membrane and peptidoglycan-associated stresses, regulating the expression of genes involved in virulence, capsule biosynthesis and motility in many *Enterobacteriaceae*. While capsule biosynthesis and motility-associated structures were lost during the evolution of the enteropathogen *Shigella* from commensal *Escherichia coli*, *Shigella* retained the Rcs system, suggesting its potential relevance in pathogenicity. By constructing mutants lacking and overexpressing the components of the system, we assessed the importance of Rcs in response to different environmental stimuli bacteria may encounter while infecting host cells. Additionally, we examined the involvement of Rcs in intracellular survival and invasion of THP-1-derived macrophages and Caco-2 epithelial cells. Our data indicate that a perturbation of Rcs signalling affects bacterial resistance to environmental stress and antimicrobial compounds, intracellular survival and cell invasion, all properties required for a successful infection. Our work provides initial insights into the role of the Rcs system in *Shigella* pathogenesis, suggesting that this conserved phosphorelay may contribute to the bacterium's adaptation to the intracellular niche.

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C23

The Impact of Nucleotide Composition on Gene Expression in *Wolbachia pipientis*: a mechanism regulated by the host?F. Comandatore¹, S. Papaleo¹, S. Panelli¹, I. Bitar², L. Sterzi¹, R. Nodari³¹Department of Biomedical and Clinical Sciences, Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Italy²Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czechia³Istituto Nazionale di Genetica Molecolare (INGM) "Romeo and Enrica Invernizzi", University of Milan, Italy

Wolbachia pipientis is an obligate intracellular bacterium found in numerous arthropods and filarial nematodes. It engages in diverse symbiotic relationships with its hosts, which has led to the loss of many genes and regulatory elements. Despite this genomic reduction, experimental evidence indicates that *Wolbachia* gene expression remains coordinated with host physiology, although the underlying regulatory mechanisms are not fully understood.

In this study, we analyzed publicly available RNA-seq datasets from four *Wolbachia* strains—*wOo*, *wDi*, *wBm*, and *wMel*—and found that nucleotide composition significantly correlates with gene expression. However, this effect varied across samples and strains, with its magnitude and direction correlating with the expression levels of the SAM-dependent methyltransferase gene, *midA*. Notably, *midA* overexpression was associated with a negative correlation between gene adenine content and expression, whereas *midA* downregulation reversed this trend.

MidA is known to methylate arginine residues on proteins, potentially altering their affinity for substrates such as nucleic acids. To further investigate the function of this poorly characterized enzyme, we expressed *Wolbachia midA* in *Escherichia coli* and found that it can also methylate both adenine and cytosine in DNA. Additionally, upstream of the *midA* gene, we identified a conserved binding site for the Ccka/CtrA signal transduction system, a pathway likely involved in host–bacterium communication.

Collectively, these findings suggest a regulatory cascade wherein host signaling activates the *Wolbachia* Ccka/CtrA system, inducing *midA* expression and subsequently modulating the expression of multiple *Wolbachia* genes in a nucleotide composition-dependent manner.

C24

Systematic Targeting of GD2-positive Neuroblastoma Tumors with Photoconcolytic Phage NanobotsA. Danielli^{1,4}, S.K. Zadrán^{1,4}, N. Facchinello¹, P. De Rosa¹, R. Saporetti², P.E. Costantini^{1,4}, L. Ulfo¹, M. Nigro^{1,4}, A. Petrosino¹, L. Pappagallo¹, S. Aloisi¹, G. Milazzo¹, Z. Abe Din¹, A. Rigamonti¹, L. Flora¹, M. Santulli¹, L. Cimadom¹, G. Zuccheri¹, M. Zangoli³, M. Di Giosia^{2,4}, F. Di Maria³, R. Bernardoni¹, M. Calvaresi^{2,4}, G. Perini^{1,4}¹Department of Pharmacy and Biotechnology Alma Mater Studiorum – University of Bologna, Italy²Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum – University of Bologna, Italy³The Institute for Organic Synthesis and Photoreactivity, National Research Council, Bologna, Italy⁴IRCCSAOUBO Sant'Orsola–Laboratory of Preclinical and Translational Research in Oncology (PRO), Bologna, Italy

Neuroblastoma (NB) is one of the most common pediatric neuroendocrine tumors originating from neural crest-derived sympathoadrenal precursor cells and accounts for 15% of pediatric cancer mortalities. Disialoganglioside-GD2 is a key molecular target for NB immunotherapy which is based on the employment of GD2-targeting antibodies. However, about 50% of treated patients can experience tumor relapse due to limited immune-mediated cytotoxicity and poor antibody penetration into tumors. To address this problem, we developed a tumor-

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penetrating photooncolytic phage nanovector platform that selectively targets GD2-expressing NB cells. The phage bioconjugates were functionalized with different photosensitizers, and resulted in targeted oncolysis of GD2-positive NB cells upon light irradiation, without affecting GD2-negative ones. The photooncolytic phage nanobots were shown to deeply penetrate into GD2-positive tumor spheroids in vitro, and to cross biological barriers in a Zebrafish xenograft model, maintaining their ablation specificity upon irradiation. Finally, to counter resistance from GD2 loss, often linked to poor prognosis, we introduced a CRISPRa strategy to reactivate GD2 expression in GD2-negative cells. This approach offers a minimally invasive and highly effective strategy, addressing unmet needs in Neuroblastoma therapy.

C25	Antitumor activity of <i>Lactococcus lactis</i> cell-free supernatant on human glioblastoma cell lines
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In the last few years, probiotics have gained much attention within the medical, pharmaceutical, and food fields, given the health benefits provided by their consumption. They include several lactic acid bacteria (LAB) species, mostly belonging to the genera *Lactobacillus*, *Lactococcus*, and *Streptococcus*. Postbiotics are bioactive compounds (organic acids, short-chain fatty acids, enzymes, and neurotransmitters) produced by bacterial fermentation that exert different health effects. It is well known that probiotics, as health-promoting microorganisms, show different therapeutic properties, including anti-pathogenic, anti-inflammatory, and cholesterol-lowering activities. Recently, accumulating evidence has shown that certain commensal bacteria play protective roles against cancer; thus, anti-carcinogenic activity is one of the most interesting probiotics properties that is currently under investigation. Here, we studied the anticancer properties of postbiotics produced by three different *Lactococcus lactis* subsp *lactis* strains isolated from natural whey starter cultures on human glioblastoma cell lines. MTT and Trypan Blue exclusion assays revealed a significant reduction in cell proliferation, and flow cytometry analysis corroborated this data, demonstrating a cell cycle arrest in treated cells. Moreover, other cancer hallmarks, such as wound healing rate closure and migration, were markedly inhibited by postbiotics. On the other hand, primary astrocytes viability and the blood-brain barrier (BBB) integrity were not impaired, suggesting a selective effect of postbiotics on proliferating-undifferentiated cells. This preliminary study highlights, for the first time, the potential anticancer properties of postbiotics from some *L. lactis* strains on human glioblastoma cell lines.

C26

Deletion of the *MSMEG_0394* gene in *Mycobacterium smegmatis* affects cell surface properties

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Non-tuberculous mycobacteria (NTM) infections are rising globally and remain difficult to treat due to intrinsic resistance to antibiotics, largely attributed to the low permeability of their lipid-rich cell envelope. Glycopeptidolipids (GPLs), a major class of glycolipids present in the outer layer of many NTM species (e.g. *Mycobacterium abscessus*, *Mycobacterium smegmatis*), play an important role in both mycobacterial physiology and pathogenicity. We previously identified the *MSMEG_0394* gene within the conserved GPL biosynthetic cluster in *M. smegmatis*. The predicted protein product shares 75% amino acid identity with the *M. abscessus* *MAB_4102c* gene product, both of which are annotated as "uncharacterized proteins." To investigate whether the *MSMEG_0394* gene product is involved in GPL biosynthesis, we isolated an *M. smegmatis* mutant strain carrying a null mutation in *MSMEG_0394*. Here, we report phenotypic analyses of the mutant and wild-type strains, revealing significant alterations in cell surface properties, including rough colony morphology, changes in biofilm architecture and drug resistance, complete loss of sliding motility, and increased autoaggregation. Moreover, *in silico* structural analysis of the predicted *MSMEG-0394* protein excluded DNA-binding and membrane-associated roles, indicating instead a cytosolic localization and a potential role in protein-protein interactions. As part of ongoing work, the Δ *MSMEG_0394* strain will be evaluated for susceptibility to various host-relevant stresses such as acid-nitrosative stress and lysozyme stress, which mimic the intracellular environment encountered within macrophages. Additionally, extended lipid profiling is underway to further elucidate the impact of *MSMEG_0394* deletion on cell envelope composition. Studying GPL-deficient mycobacterial mutants is crucial for identifying new antimicrobial targets for therapeutic development.

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C27

Free-living amoebae as a potential reservoir of Herpes Simplex VirusN. Diaz¹, A. Congiargiu¹, V. Margarita¹, P. Rappelli¹, P.L. Fiori¹, L.A. Sechi¹, S. Jasemi¹, C. Cacciotto², A. Alberti², D. Dessì¹¹*Department of Biomedical Sciences, University of Sassari, Italy*²*Department of Veterinary Medicine, University of Sassari, Italy*

Free-living amoebae (FLA) are ubiquitous protists found in a variety of habitats. FLAs are of considerable importance in two apparently unrelated fields: the ecology of microbial communities, and human and animal health. FLA feed by phagocytosis on various microorganisms, and therefore play a pivotal role in shaping microbial communities.

The impact of FLA on human and animal health is related to both its capability to cause infections and to act as a reservoir and as a vector of several pathogenic microorganisms. Several species have evolved mechanisms to survive phagocytosis by FLA, and therefore may play a role in the transmission and shedding of microbial infections.

Interestingly, a FLA species (*Acanthamoeba castellanii*) and a virus (Herpes Simplex Virus – HSV) can both independently cause keratitis in humans. Coinfection by these two organisms has also been reported; however, to our knowledge, the presence of HSV-1 as a component of the *Acanthamoeba* virome has not been documented. The aim of the present study was to determine

whether *Acanthamoeba* could play a role in the survival and transmission of HSV-1. We followed viral persistence by coculturing HSV-1 and *Acanthamoeba*. Cells samples were collected at 3, 7 and 30 days post-infection and real time PCR, immunofluorescence and virus titration on permissive cells were performed. We observed an increase in the multiplication capacity of infected amoebae after 24 hours of infection with HSV-1. To our knowledge, this is the first study suggesting that FLAs can act as reservoirs and vectors of HSV-1, potentially contributing to its spread.

C28

The Multifunctional Role of Extracellular Vesicles from *Lactocaseibacillus rhamnosus* GG

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Bacterial extracellular vesicles (bEVs) are nano-sized, lipid membrane-delimited particles secreted by both Gram-negative and Gram-positive bacteria. They represent a key bacterial secretion pathway due to their distinct biological composition compared to their parent cells. Despite increasing interest, bEVs from Gram-positive bacteria, such as *Lactocaseibacillus rhamnosus* GG (LGG), remain less explored, mainly due to the challenges posed by their thick cell walls. However, their ability to deliver bioactive molecules makes them key mediators of host-microbe communication. This study aimed to isolate bEVs from LGG and investigate their role in probiotic mechanisms. Using ultrafiltration, size- and density-based separation methods, we obtained pure LGG EVs which were characterized following MISEV 2023 recommendation, employing electron microscopy (EM), nanoparticle tracking analysis (NTA), and a TLR-2 based reporter assay.

Functional studies demonstrated that LGG EVs protect intestinal epithelial integrity by reducing LPS-induced disruption of tight junctions in Caco-2 monolayers. Furthermore, they dampened neuroinflammatory response in LPS-stimulated BV2 microglial cells, suggesting a modulatory role in gut-brain axis communication. To uncover molecular drivers of these effects, proteomic profiling via LC-MS/MS identified over 400 proteins associated with LGG EVs, including chaperones, immune modulators, RNA-binding proteins, metabolic enzymes and factors involved in epithelial homeostasis.

Altogether, our data reveal LGG-derived EVs as active vehicles of probiotic function, contributing to epithelial protection and immune modulation. Their defined protein cargo offers mechanistic insight and supports their potential as novel therapeutic tools in inflammatory and neuroimmune disorders.

C29	Inhibition of SARS-CoV-2 RNA Polymerase by Suramin Derivatives: Biochemical and Antiviral Characterization
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The COVID-19 pandemic, caused by SARS-CoV-2, first appeared in Wuhan, China, in late 2019 and rapidly evolved into a global crisis, leading to over 7 million deaths and causing major disruptions to public health systems and economies worldwide. Central to the viral replication machinery is the RNA-dependent RNA polymerase (RdRp) nsp12, which functions alongside the cofactors nsp7 and nsp8 to drive the synthesis of the viral single-stranded positive-sense RNA genome. Despite ongoing efforts, only three antiviral agents have been approved for treating COVID-19, and their clinical performance remains modest. Suramin—a polysulfonated compound originally introduced by Bayer for treating African trypanosomiasis—has recently shown strong antiviral activity against SARS-CoV-2, both by inhibiting RdRp in vitro and suppressing viral replication in cell-based assays. In addition to its antiparasitic properties, suramin has a diverse pharmacological profile, including antiviral and anticancer activities. In this study, we explored a library of suramin-derived molecules with the goal of identifying novel RdRp inhibitors. These analogs were assessed through biochemical and cellular assays to evaluate their antiviral activity. Through structure–activity relationship (SAR) analysis, we identified key molecular features that contribute to improved potency. This led to the discovery of new derivatives with nanomolar-level inhibition of RdRp and effective antiviral activity in the low micromolar range in infected cells, offering valuable insights for the design of advanced therapeutics targeting SARS-CoV-2.

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C30	Uncovering New HCMV Genes Involved in APOBEC3B Relocalization Using a CRISPR/Cas9- Immunofluorescence Screening
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The APOBEC3 (A3) family is primarily known for its role in restricting DNA-based viruses. Among the human A3 enzymes, APOBEC3B (A3B) is unique in being predominantly localized in the nucleus. Previous studies have shown that herpesviruses such as HSV-1, EBV, and KSHV use the large subunit of their ribonucleotide reductase (RNR) to relocate A3B from the nucleus to the cytoplasm, thereby protecting their viral DNA. In this study, we found that the beta-herpesvirus HCMV also causes A3B relocalization, but through a novel mechanism that does not involve RNR. To identify the viral factor(s) responsible, we developed a CRISPR/Cas9-based genome editing approach combined with immunofluorescence (IFA) to target specific regions of the HCMV genome and observe changes in A3B localization. Because A3B relocalization occurs early during infection, we saw reduced cytoplasmic A3B levels when genes involved in immediate/early gene transcription were knocked out. Notably, deletion of a specific early gene completely blocked A3B relocalization. Disruption of known regulatory elements of this gene also led to reduced relocalization, reinforcing its central role. Follow-up experiments confirmed this gene's importance and suggested that additional early genes may work together in a complex to drive A3B relocalization during HCMV infection.

C31	Investigating Human Gut Microbiota-Host Interactions Using a Defined <i>In Vitro</i> Gut Microbiota Model in the Presence of FOS and Probiotics
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The human gut microbiota (HGM) is a complex ecosystem mainly composed of five bacterial phyla (Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota, and Verrucomicrobiota) and plays a fundamental role in host physiology. It contributes to key functions, including digestion, metabolism, immune system modulation, and protection against pathogens. These functions are primarily mediated by bacterial metabolites, including short-chain and branched-chain (SCFAs and BCFAs), bacteriocins, and exopolysaccharides (EPS).

This study employs a defined *in vitro* gut microbiota model consisting of a minimal core community supplemented with probiotic strains to investigate microbial interactions and their effects on the host. The model is tested using fructooligosaccharides (FOS, DP~10) as a carbon source. All probiotic strains are able to metabolize FOS. Genes potentially involved in FOS utilization were identified in these strains.

To evaluate the functional impact of the produced probiotics metabolites, supernatants from FOS fermentations are tested on HepG2 liver cells in which metabolic dysfunction-associated fatty liver disease (MAFLD) is induced using palmitic acid. The treatment results in a measurable reduction in intracellular lipid accumulation.

These preliminary findings suggest that specific probiotic strains, through FOS fermentation and production of beneficial metabolites, may contribute to the modulation of gut-liver axis pathways and offer promising perspectives for improving MAFLD-related conditions.

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C32	The stringent response is a key determinant of <i>Pseudomonas aeruginosa</i> intracellular survival in A549 airway epithelial cells
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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen known for causing persistent, life-threatening infections and exhibiting antibiotic resistance. Traditionally regarded as an extracellular pathogen, it can invade and survive within both professional and non-professional phagocytic cells, enabling it to evade host immune responses and antimicrobial treatments.

This study investigates the role of the stringent response (SR), a global regulatory system involving the second messenger (p)ppGpp and the DksA protein, in the *P. aeruginosa* survival within A549 human carcinoma airway epithelial cells.

P. aeruginosa mutants inactivated in the SR (unable to synthesize (p)ppGpp and/or lacking DksA) were generated and phenotypically characterized. Intracellular survival of the wild-type PAO1 strain and its derivative SR mutants within A549 was assessed using CFU counts and confocal microscopy, including experiments in the presence of bafilomycin A1, a vacuolar acidification inhibitor. The impact of SR on Type III Secretion System (T3SS) expression during infection was also evaluated by using transcriptional fusions. Results show that *P. aeruginosa* can survive inside A549 cells for up to 24 hours, whereas SR mutants exhibit significantly reduced intracellular

survival and impaired evasion of vacuolar acidification relative to the wild-type strain. Reporter assays revealed that SR positively regulates T3SS expression, with SR mutants showing reduced T3SS effector expression. These findings indicate that the SR is critical for *P. aeruginosa* survival within A549 cells, at least in part through regulation of T3SS, and highlight its potential as a therapeutic target for tackling *P. aeruginosa* chronic infections.

C33 Interspecies cross-talk among key infant gut microbiota members

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In vitro gut models allow controlled investigation of microbial interactions outside the host environment. To study interspecies cross-talk within the infant gut microbiota, representative strains of *Bacteroides fragilis*, *Bacteroides uniformis*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* subsp. *longum*, were selected from a curated biobank based on high species-representative scores. Strains were cultivated in monoculture and in pairwise combinations using a validated infant gut-simulating medium under anaerobic conditions. Growth was assessed after 8 hours by qPCR, measuring log₁₀ fold change relative to the initial inoculum. Results show that *B. bifidum* and the *Bacteroides* strains did not exhibit enhanced growth in co-culture, consistent with their limited co-occurrence *in vivo*. In contrast, *B. longum* showed increased growth when paired with *B. bifidum* and stimulated both *B. bifidum* and *B. fragilis*, indicating a supportive role. To assess molecular responses, a cross-talk index was calculated based on differentially expressed genes compared to monocultures. The strongest transcriptional modulation occurred in the *B. bifidum*–*B. longum* pair. *B. longum* also showed extensive transcriptional changes in the presence of *B. fragilis* and *B. uniformis*, particularly in genes related to carbohydrate and amino acid metabolism, while *B. fragilis* responded minimally. These findings indicate that *B. longum* actively promotes interspecies interactions, supporting both growth and metabolic activity of other taxa. This highlights its central role in shaping the infant gut microbiota. Based on this evidence, the cultivation of synthetic microbiotas has been initiated in scalable bioreactor systems, enabling functional validation and further exploration of community assembly during early microbiome development.

C34 Enrichment and characterization of Candidate Phyla Radiation bacteria from human and environmental habitats

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Candidate Phyla Radiation (CPR) represents a diverse and largely uncultivated group of bacteria that plays a significant yet still poorly understood role in both human and environmental microbiomes. Despite their genetic and ecological diversity, many CPR exhibit unique adaptations, such as reduced genome sizes, unconventional metabolic pathways, and

symbiotic/parasitism relationships with other microorganisms. These interactions can influence the expression of specific metabolic genes in the host microorganism, affecting physiological processes, including nutrient metabolism, immune function, and host-microbe co-metabolism. Some CPR also appear to harbour resistance genes and exhibit adaptive strategies to survive in challenging environments. In this study, we cultivated and characterized CPR from several human and environmental ecosystems, including vaginal and nasal environments, broad bean roots, and contaminated peat-rich aquifers. The bacterial host cells employed for cultivation/enrichment included either reference actinobacterial strains or other bacterial strains isolated from each ecosystem. Molecular characterization revealed distinct CPR taxa associated with each ecosystem. Co-cultivation procedures with actinobacterial host cells were particularly successful in enriching CPR, which belonged to Candidatus (Ca.) Saccharibacteria, Ca. Nomurabacteria, and Ca. Roizmanbacteria. However, human-derived *Lactobacillus* host strains were less effective in hosting CPR, suggesting group-specific symbiotic relationships. Finally, preliminary functional assays indicated that CPR interaction reduced antibiotic production in one actinobacterial host. The study of CPR is reshaping our understanding of evolutionary processes, offering novel insights into microbial resilience, the evolution of resistance traits, and the re-evaluation and improvement of antimicrobial strategies.

C35

Diet-Dependent Microbial and Metabolic Signatures in Food: Implications for Cardiovascular Health

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Each meal introduces billions of mostly harmless bacteria. Food-associated microbes and their metabolites can influence disease prevention and treatment. This study presents integrative profiling of food as a novel approach for monitoring cardiovascular diseases (CVDs). To explore dietary impacts on cardiovascular health, a randomized, cross-over controlled clinical trial was conducted at Imperial College London. Hospitalized participants followed two 3-day dietary interventions: one healthy (HD: high fibers, low fats) and one non-healthy (NHD: low fibers, high fats). Composite diet samples were analyzed via 16S rRNA sequencing at the University of Milano-Bicocca, generating 8,864,768 reads. Alpha diversity was higher in NHD (464 vs 103 genera), based on Pielou and Shannon indexes. Beta diversity also differed significantly (Bray-Curtis, Jaccard). HD was dominated (relative abundance >3%) by *Janthinobacterium*, *Serratia*, and *Flavobacterium*, while NHD diet featured *Bacillus*, *Photobacterium*, *Thermus*, *Anoxybacillus*, and *Geobacillus*. *Pseudomonas* and *Lactococcus* were prevalent in both. Over 50 genera differed significantly between diets, with ASVs from the same genus often showing opposing trends. Metabolic profiling (PLS-DA) revealed 39 significantly different metabolites, including SCFAs. Acetate was associated with 7 genera in HD; propionate was positively associated with *Paraburkholderia* and negatively with *Romboutsia*. In NHD, 9 genera were negatively correlated with propionate and 38 with acetate. These findings highlight the value of examining both microbial and metabolic composition, particularly SCFAs, in assessing dietary health effects. The diet-dependent microbial and metabolic signatures suggest a potential mechanism linking diet to CVD risk, supporting the development of microbiome-informed nutritional strategies for CVD prevention and management.

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C36	Characterisation of the transcriptional regulation of the <i>mmpS6/mmpL6</i> operon in TbD1-intact <i>Mycobacterium tuberculosis</i> strains
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The deletion of the TbD1 genomic region represents the evolutionary bottleneck characterising the globally spread *Mycobacterium tuberculosis* (*Mtb*) lineages in comparison with *Mtb* lineages restricted to specific geographic areas (South East Asia or East Africa). TbD1-deletion correlates with increased resistance to oxidative stress and hypoxia, and enhanced virulence in animal models. The TbD1 locus encompasses the *mmpS6-mmpL6* operon, which in TbD1-intact strains encodes for members of the MmpS/MmpL mycobacterial membrane protein families. To date, the molecular mechanisms regulating the expression of the *mmpS6-mmpL6* operon are still unknown. *In silico* predictions and experimental evidences suggest that the operon might be regulated by two Tet-R transcriptional repressors: the Rv1556/MTB36_2740023 and Rv0302/MTB36_540006 regulators (in *Mtb* reference or TbD1-intact Tb36 strains, respectively). In this study, the impact of MTB36_2740023 and MTB36_540006 on *mmpS6-mmpL6* expression was investigated. The MTB36_Δ0023 and MTB36_Δ0006 mutants, deleted for the MTB36_2740023 and MTB36_540006, respectively, were constructed. Analysis of the *mmpL6* gene expression profile revealed an increased expression of the *mmpL6* gene only in the MTB36_Δ0023 mutant. Consistently, MTB36_Δ0023 showed a reduced survival after exposure to hydrogen peroxide *in vitro* as compared to the MTB36_Δ0006 mutant and the wild-type strain.

These findings indicate that MTB36_2740023 is the major regulator of the *mmpS6-mmpL6* operon, at least in standard nutrient-rich media, at optimal oxygen concentrations. Further expression and survival assays in different experimental conditions (e.g. hypoxia) will contribute to clarify the *mmpS6-mmpL6* transcriptional network, thus contributing to the elucidation of the patho-adaptation mechanisms of the globally spread TbD1-deleted *Mtb* strains.

C37	Comprehensive analysis of human endogenous retroviruses (HERV) transcriptome in SARS-CoV-2 acute infection: peculiarities and common signatures with other COVID-19 stages
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Human Endogenous Retroviruses (HERV) can trigger innate immune activation in infectious and inflammatory contexts, due to the expression of immunogenic transcripts and proteins. In turn, SARS-CoV-2 infection stimulates an imponent inflammatory response, which characterizes COVID-19 pathogenesis. Since both viral infection and inflammation are reported to modulate HERV expression, HERV activation in COVID-19 can contribute to its clinical manifestations.

To give an understanding on this interplay, we performed the high-throughput sequencing of ~3300 HERV loci in the peripheral blood mononuclear cells (PBMC) of 37 individuals with acute SARS-CoV-2 infection and 8 healthy controls (HC). PBMC have been chosen as these cells are not directly infected by the virus but have a crucial role in the inflammatory and immune events defining COVID-19 pathogenesis.

Results showed that HERV expression is modulated by SARS-CoV-2 and clearly divide infected individuals from HC in clustering analyses. Differential expression analyses confirmed that 359 HERV loci were upregulated (253) or downregulated (106) in the presence of SARS-CoV-2 infection. Such transcriptional signature has been compared with the one from convalescent and re-testing positive patients, revealing a specific pattern of expression during primary infection but also a subset of HERVs significantly modulated in all COVID-19 clinical stages that were frequently colocalized with cellular genes with a role in antiviral immunity or cellular processes relevant for COVID-19 pathogenesis.

Overall, the present study shows a comprehensive picture of HERV transcriptome in PBMC and its modulation in different COVID-19 clinical stages, identifying specific elements with possible relevance for the disease manifestation and outcome.

C38

Intracellular and extracellular potential of homoisoflavone derivatives against *Mycobacterium tuberculosis*

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Tuberculosis (TB) is the leading cause of death from infectious diseases worldwide. Current treatment still has limitations, mainly related to its long duration, severe adverse effects, and the development of bacterial resistance mechanisms. Due to these factors, research into new anti-TB drugs is necessary and urgent. Homoisoflavones, a unique subgroup of flavonoids characterized by their 3-benzylidenechroman-4-one skeleton, were investigated previously by our group regarding their antimycobacterial activity, and three derivatives stood out. Initially, in the present study, these most promising derivatives had their MIC (Minimum Inhibitory Concentration) determined by the REMA (Resazurin Microtiter Assay), a widely used and reliable method. Derivatives **19**, **20**, and **21** significantly inhibited the *Mycobacterium tuberculosis* (Mtb) H37Rv growth (MIC 2.5, 0.3, and 40 µM, respectively) and showed good selectivity index when the cytotoxicity was evaluated on THP-1 derived macrophages. These derivatives showed MBC (Minimum Bactericidal Concentration) values equal to the MIC, confirming their bactericidal activity. Furthermore, these derivatives inhibited intracellular Mtb H37Rv growth in infected THP-1-derived macrophages, especially derivative **20** (4 x MIC: 1.2 µM). In addition, to identify the mechanism of action of these compounds, resistant mutants will be isolated by plating Mtb H37Rv cultures on 7H10 agar plates containing the compounds (5–20 fold MIC). Moreover, to identify the polymorphism that could be responsible for the observed resistance phenotype, whole genome sequencing (WGS) of the new isolated resistant mutants will be performed. Taken together, these results will contribute to the characterization of these compounds as antitubercular drug leads.

C39	Production and characterization of a phage-encoded capsular polysaccharide endoglycosidase targeting Sequence Type 307 <i>Klebsiella pneumoniae</i> strains
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Bacteriophages are viruses that infect and lyse bacterial cells by using several types of specialized proteins. Among these, endoglycosidases (EGs) target and degrade capsular polysaccharides (CPS), key virulence factors protecting bacterial cells. Here we characterized a phage-derived endoglycosidase (EG-GP1) able to specifically target and degrade the CPS expressed by an emerging high-risk clone of multidrug-resistant (MDR) *Klebsiella pneumoniae*.

Bioinformatics analysis of the genome of the vB_KpS_GP-1 phage, able to lyse Sequence Type (ST) 307 *K. pneumoniae* strains, revealed the presence of two putative endoglycosidase-encoding genes (*orf57* and *orf58*). The latter, encoding a protein showing 77.4% identity with a previously characterized endoglycosidase, was selected for heterologous expression in *Escherichia coli* by using a N-terminal His-Tag cloning strategy.

The EG-GP1 recombinant protein was produced in soluble form and its activity was proved by spot-test on the vB_KpS_GP-1 indicator strain. SDS-PAGE and western-blot revealed a band of ≈135 kDa, in line with theoretical calculations. EG-GP1 tested positive by spot-test for 11/14 (79 %) ST307 strains, showing results in agreement with vB_KpS_GP-1 host-range, i.e. being inactive on capsule-deficient ST307 strains. In addition, EG-GP1 tested negative also on strains belonging to other STs (*n*=7). Negative-stain microscopy showed a statistically significant reduction of extracellular area of EG-GP1-treated cells of the indicator strain when compared to untreated, while no significant changes were observed by using *K. pneumoniae* ATCC 13883. To summarize, EG-GP1 may represent a possible additional therapeutic tool to be combined with standard or novel antibiotics against infections by MDR ST307 *K. pneumoniae* strains.

C40	MetE (Rv1133c): a secreted immunogenic antigen of <i>Mycobacterium tuberculosis</i> that may represent a new marker of active replication
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To identify new secreted antigens with potential immunodiagnostic power we immunized mice with sterile culture medium supernatants of *Mycobacterium tuberculosis* (Mtb); with this procedure we obtained several monoclonal antibodies (mAbs), two of which bound and immunoprecipitated an 80-kDa protein that was identified by mass spectrometry as Rv1133c, the methionine synthase MetE. The protein MetE is ubiquitous among prokaryota and shows a significant sequence homology in many bacteria. We produced both the full-length recombinant (r) MetE and its N-terminal fragment, whose sequence is more conserved among mycobacteria, to select mAbs recognizing an Mtb-specific region of MetE. Finally, we produced and selected eight mAbs that specifically detect the MetE protein in the supernatant and cell lysate of Mtb and BCG, but

not other bacteria such as non-tuberculous mycobacteria (NTM), *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, or *Escherichia coli*. Taking advantage of our mAbs, we studied the vitamin B12 dependence for the synthesis of MetE in Mtb and NTM as well as the kinetics of MetE production and secretion in supernatants during the *in vitro* reproduced replicative, dormant, and resuscitation cycle of Mtb. Our data demonstrate that dormant Mtb bacteria, which are assumed to be prevalent in latent infections, as well as NTM do not produce and secrete MetE. Results indicate an unexpected specificity for Mtb of our anti-MetE mAbs and encourage the use of rMetE and our mAbs as tools for the immunodiagnosis of tuberculosis and its stages.

C41	Microbiota Matters: Investigating Bacterial Responses to Chemotherapeutic Agents in Ovarian Cancer
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Ovarian cancer remains a significant global health challenge, with emerging evidence highlighting the role of tumor-associated microbes in cancer progression and treatment, underscoring the importance of pharmacomicrobiomic studies in understanding drug-microbiota interactions. This study evaluated the effects of carboplatin, paclitaxel and doxorubicin on ovarian cancer cell lines (SKOV3, Ovar3, Kuramochi), a normal cell line (FT190), patient-derived organoids, and newly isolated tumor-associated bacteria to contribute to the personalized therapeutic approaches. Minimum Inhibitory Concentrations for standard antibiotics (ampicillin, tetracycline, gentamicin, vancomycin) were also determined for each characterized bacterial strain. Results of cytotoxicity testing revealed differential sensitivities on cell lines, with doxorubicin demonstrating the highest potency, reducing cell survival to less than 10% in all cell lines however, in patient-derived organoids the response was more variable. Reference bacterial strains (*E. coli*-25922 and *L. rhamnosus*-GG) and most tumor-associated isolates (including *E. coli*, *S. pneumoniae*, *S. constellatus*, *P. aeruginosa*, *K. michiganensis*, *L. rhamnosus*, *L. paracasei*) showed resistance to chemotherapeutic agents. However, two *L. rhamnosus* and *Streptococcus* isolates were notably sensitive to doxorubicin (50 µM) under both aerobic and anaerobic conditions, while *L. paracasei* responded only in aerobic conditions. *K. michiganensis* was uniquely susceptible to all drugs under anaerobic conditions. Antibiotic testing revealed varied susceptibility patterns among the bacterial isolates, with some showing resistance. Notably, reference strains tended to be more susceptible compared to patient-derived isolates. These findings highlight that chemotherapeutic agents effectively target cancer cells but show limited impact on tumor-associated bacteria, potentially influencing cancer progression. Future studies should explore integrating microbiota-targeted strategies to enhance chemotherapy efficacy.

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C42	AggR repression by medium-chain fatty acids reduces biofilm and virulence in Enteroaggregative <i>Escherichia coli</i> 17-2
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Enteroaggregative *Escherichia coli* (EAEC) is a leading cause of acute and persistent diarrhea worldwide, especially in children. A key feature of EAEC pathogenesis is the formation of a thick biofilm on the intestinal mucosa, driven by the transcriptional activator AggR, a member of the AraC/XylS family, which regulates the expression of several virulence-related genes, including aggregative adherence fimbriae (AAF). This study investigates the anti-virulence effects of fatty acids (FAs) against the prototypical EAEC strain 17-2, focusing on their potential to interfere with the regulation mediated by AggR.

Growth assays demonstrated that treatment with a pool of medium- and long-chain FAs at 0.02% does not affect bacterial viability. Subsequent analysis on biofilm formation revealed that three medium-chain FAs - caprylic (C8), lauric (C12), and decenoic (C10:1) acids - significantly decrease biofilm biomass by approximately 70%. To explore the molecular mechanism, we evaluated the expression profile of *aggR* and *aggA*, showing a consistent downregulation at both transcriptional and protein levels upon treatment with each FA. Infection assays using HEp-2 cells further confirmed the phenotypic effects of FA treatment, showing reduced bacterial adherence and cytotoxicity. These findings support a model in which specific FAs reduce EAEC pathogenicity by interfering with the expression of its master regulator AggR, likely through a transcriptional mechanism.

Ongoing research is investigating whether this regulation involves bacterial fatty acid sensing systems, such as FadD, or other upstream modulators of *aggR*. Targeting these regulatory pathways may offer novel therapeutic strategies against EAEC infections.

C43	A novel membrane-targeting agent against pathogenic Firmicutes
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A key strategy to counteract the spread of antimicrobial resistance involves discovering novel antimicrobial compounds. Historically, natural products have been the most significant source, yielding approximately 60% of all new antibacterial chemical entities. Here, we describe a novel antimicrobial agent identified through a whole-cell phenotypic screening of 113 plant-derived compounds against a collection of Gram-negative and Gram-positive bacteria. A structurally defined compound extracted from Leguminosae plants, consisting of an isoprenoid-substituted benzoic acid derivative (named ISBAD1), effectively inhibited the growth of both methicillin-susceptible and resistant *Staphylococcus aureus* strains (MIC=4 mg/L; MBC=16 mg/L). Interestingly, ISBAD1 showed comparable activity against Firmicutes (*i.e.*, *Enterococcus* spp., *Listeria monocytogenes*, and *Bacillus spizizenii*), while it had no effects on Actinobacteria (*i.e.*, *Mycobacterium abscessus* and *Mycobacterium smegmatis*) or Gram-negative pathogens (*i.e.*, *Acinetobacter baumannii*,

Escherichia coli, and *Pseudomonas aeruginosa*). ISBAD1 also inhibited biofilm formation and disrupted a preformed biofilm of *S. aureus*. Notably, ISBAD1 has a low toxicity profile in mice and significantly increased the survival of *Galleria mellonella* larvae infected with a lethal dose of *S. aureus*. Investigations on the ISBAD1 mechanism of action revealed that this compound selectively perturbs *S. aureus* membrane integrity, while it had no cytolytic or cytotoxic effects on human cells. Accordingly, as observed for other membrane-disruptive compounds, the frequency of spontaneous resistant mutants of *S. aureus* was lower than that of other standard-of-care antibiotics such as vancomycin, oxacillin, and linezolid. These data suggest that ISBAD1 could further be developed as a promising drug for difficult-to-treat infections caused by pathogenic Firmicutes.

C44 Respiratory microbiota signatures associated with ventilator-associated pneumonia

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Ventilator-associated pneumonia (VAP) is a frequent complication in mechanically ventilated patients, yet the role of the respiratory microbiota in the etiology of this condition remains not understood. This study aimed to longitudinally characterize the respiratory microbiota in a matched cohort of critically ill patients to identify microbial signatures potentially associated with VAP development

A total of 146 intubated adult patients were enrolled across multiple intensive care units, forming a matched cohort of 73 patients who developed VAP and 73 no-VAP controls. Oropharyngeal swabs (TOF) and bronchial aspirates (BAS) were collected at intubation (T0) and either at the time of VAP diagnosis or at a matched timepoint in controls (TVAP). Microbial composition was assessed through 16S-rRNA gene sequencing.

Microbiota profiling revealed substantial inter- and intra-individual variability across timepoints and sampling compartments. While a modest reduction in microbial richness was observed in VAP patients at baseline, compositional shifts assessed through beta-diversity metrics indicated notable temporal changes within individuals. Furthermore, taxonomic analysis showed that genera such as *Corynebacterium*, *Kocuria*, and *Mycoplasma* were statistically more abundant in no-VAP patients, whereas *Escherichia-Shigella*, *Peptoniphilus*, and *Gemella* were enriched in VAP samples. Additionally, cytokine profiling identified correlations with specific taxa, suggesting potential immunomodulatory interactions.

These findings support a possible contribution of the respiratory microbiota to VAP susceptibility. However, the marked temporal and inter-individual variability suggests that multifactorial drivers, including extrinsic factors beyond the host, could shape microbial dynamics.

C45

Induction of persistent and viable but non-culturable *Pseudomonas aeruginosa* cells by cefiderocol exposure in biofilms and a murine infection model

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Persistent and viable but non-culturable (VBNC) *Pseudomonas aeruginosa* cells impair the eradication of the biofilm-related cystic fibrosis (CF) lung infections. The siderophore-cephalosporin cefiderocol, which exploits bacterial iron-uptake systems, represents a valuable therapeutic option; however, there is limited information about its activity against bacterial persistence.

P. aeruginosa biofilms developed in iron-limited conditions were exposed to the cefiderocol minimum biofilm eradication concentration and characterized for their carriage of survivors by a combination of cultural, qPCR, and confocal microscopy analysis, to detect both culturable persisters and VBNC cells. Biofilms were challenged after 14 days of maintenance in nutrient-starvation conditions coupled with either cefiderocol, tobramycin, or ceftazidime subinhibitory concentrations.

A higher amount of culturable *P. aeruginosa* persisters was recovered after cefiderocol challenge (2×10^5 CFU/ml) compared to tobramycin and ceftazidime (between 5 and 9×10^3 CFU/ml); consistently, a lower VBNC cell percentage was induced by the siderophore-cephalosporin (88.10% vs 99.10% and 97.40% after tobramycin- and ceftazidime-exposure, respectively). Bacterial dormancy was confirmed by the detection of specific markers in confocal microscopy assays. The exposure to subinhibitory drug concentrations did not increase the amount of cefiderocol-induced VBNC cells.

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Preliminary assays in a murine *P. aeruginosa* lung infection model showed a higher bacterial survival detected by culture and a lower VBNC subpopulation (73.33%) after cefiderocol-exposure compared to tobramycin (98.05%).

Overall, these data evidence the *P. aeruginosa* ability to persist against novel and last-resort antibiotics and stress the need for considering even the role of bacterial persisters and VBNC cells in the recurrence of chronic infections, mostly in CF patients.

C46

Sex-based differences in prenatal innate immunity: divergent foetal responses to *Mycoplasma hominis* infection between males and females

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Sex differences affect the inflammatory response in the adult, with women exhibiting more active immune responses and higher pro-inflammatory cytokine secretion than men. Although these biological differences in susceptibility to infections are also reported in early life, data on sex-specific foetal immuno-response to intra-amniotic infections remain limited.

This study investigated the response of amniotic fluid cells (AFC) from male and female foetuses upon infection with *Mycoplasma hominis*, a pathogen involved in adverse pregnancy events.

Using an experimental model to investigate sex-specific innate immune responses, we infected male and female AFCs with a clinical isolate of *M. hominis*. We evaluated the gene and protein expression levels of IL-1 β , IL-6, IL-8, and TNF- α . Additionally, we compared the gene and protein expression of TLR-2 and TLR-4, the production of nitrites, and the levels of selected miRNAs in male and female AFCs infected by mycoplasmas.

The gene expression of *IL-1 β* , *IL-6*, *IL-8*, *TNF- α* , and *TLR2* were higher in infected females AFCs, while only *IL-6*, *IL-8* and *TLR4* expression were up-regulated in infected males. Protein levels of IL-6, TNF- α and TLR2 were up-regulated exclusively in infected females, who also produced higher levels of nitrites. miRNAs expression revealed an up-regulation of miR-29a-3p in infected females, and miR-223-3p in infected males, with miR-29b-3p showing up-regulation in both sexes upon infection.

Our results showed that female foetuses exhibited greater stimulated cytokines production during the mid-trimester of pregnancy than male foetuses, suggesting that differential inflammatory responses could affect maternal health and foetal development, predisposing to sex-specific foetal programming.

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C47	Cytotoxic and virological effects induced by a triorganotin derivative compound in HTLV-1 infected cells at different immortalization/ transformation stages
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Infection caused by Human T-Lymphotropic virus type 1 (HTLV-1) is characterized by life-long latency both in asymptomatic and leukemic or neurological carriers. Currently there are no effective therapies or vaccines to counteract infection or diseases caused by HTLV-1. Metal-derived platinum complexes are amply utilized for the treatment of solid tumors, but suffer from systemic toxicity and drug resistance. Recently, tin derivatives have been investigated as promising anti-cancer drug candidates. In this study the effects of a newly synthesized tin-based compound, tributyltin 2,2,2-trifluoroacetate (TBT), have been evaluated in immortalized lymphocytes generated in our labs by HTLV-1 infection *in vitro*, and rendered or not progressively independent from interleukin-2 as a growth factor, or in the C91/PL HTLV-1 transformed cell line. TBT induced a dose-dependent inhibition of metabolic activity and viability in the HTLV-1-infected cells in a cell-type-dependent fashion, being the C91/PL cells the more resistant. Investigation of cellular and molecular mechanisms involved, revealed that cytotoxicity induced by TBT could be ascribed to complex phenomena of cell death, and that caspase 3/7/8 activation, as well as apoptotic, autophagic, and pyroptotic responses seemed relevant. Molecular virology studies suggest that TBT affected also viral gene expression, especially HBZ. Taken together, the results show for the first time that an organotin-based compound can induce potent and differential effects in HTLV-1 infected cells at early and late stages of immortalization/transformation. These results can help us to better understand mechanisms of cell death resistance involved in HTLV-1 driven oncogenesis and to find novel therapeutic tools against HTLV-1.

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C48	MPXV Replication and Host Response in Vaginal and Ectocervical Epithelial Cells
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The 2022 mpox outbreak revealed the potential for sexual transmission, including in women. Replication competent virus has been detected in seminal fluid, while in female patients, vaginal lesions, vertical transmission, and miscarriage risk have been reported. This study explored the susceptibility of the lower female genital tract (LFGT) to MPXV infection, the role of sex hormones in modulating viral replication, and host-virus interactions.

Human vaginal (VK-2/E6E7) and ectocervical (Ect1/E6E7) epithelial cells were exposed to MPXV clade IIb (M.O.I. 0.1) for up to 72 hours. MPXV DNA and infectious particles were quantified. Cells were pretreated with 17-beta-estradiol (0.1, 1, 10 nM) or progesterone (1, 10, 100 nM) at physiological levels. Gene expression was analyzed by qRT-PCR and RNAseq, and MMP1 release was measured by ELISA.

Both cell lines supported productive MPXV infection. High-dose hormone pretreatment slightly reduced viral replication in Ect1/E6E7, which also showed a stronger and earlier IFN- β and IFN- λ 1 response and slower viral replication. At 48 hours post-infection, 216 differentially expressed genes (DEGs) were identified in VK-2/E6E7 and 11 in Ect1/E6E7, with 9 shared DEGs involved in protein folding (HSPA6), chemotaxis (CXCL3, ARC), inflammation and lymphoproliferation (IL11, IL1RL1, MMP1), and tissue remodeling (IGFN1, MMP1). MPXV infection significantly increased MMP1 release in both cell lines, and MMP1 inhibitors reduced infectious virus production.

These findings suggest that MPXV disrupts tissue remodeling and inflammatory pathways in the female reproductive tract, potentially affecting reproductive health, STI susceptibility, and pregnancy outcomes.

C49

Microbiome-Drug Interactions in Colorectal Cancer: Pharmacomicrobiomics and Host Dynamics

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The gut microbiome significantly impacts colorectal cancer (CRC) by affecting cancer development, progression, and treatment efficacy. Emerging pharmacomicrobiomics research highlights a two-way interaction where bacterial metabolites modulate tumor behavior and inflammation, while anticancer drugs influence microbial activity and host immunity. This study evaluated the interactions between five CRC drugs (oxaliplatin, 5-fluorouracil[5-FU], irinotecan, regorafenib, and VS1), human gut isolates, and host. Eight bacterial strains—six newly isolated from one healthy donor (three *Escherichia coli* strains and one *Enterococcus faecium* were identified) and two established commensals (*E. coli* 25922 and *Lactobacillus rhamnosus* GG)—were exposed to 5-fold drug serial dilutions, starting from 200 μ M, under aerobic and anaerobic conditions. Among the tested drugs, regorafenib and 5-FU showed antibacterial activity at 200 μ M. Regorafenib inhibited the 82% of bacterial growth of one unknown bacterial isolate, while 5-FU inhibited the 50 % bacterial growth of three isolated *E. coli* and 76% of the unknown one. Drug cytotoxicity was also assessed on two cancer (HT-29, Colo205) and one normal (MRC5) human cell lines and seven patient-derived colon organoids. Almost all drugs affected both cancer cells, with an IC50 below 4.5 μ M, except for VS1 that exhibited an IC50 value 10-times higher, and colon organoids, which exhibited variable responses depending on the drug and the patient. While almost all drugs affected cells and organoids, they had minimal impact on gut bacterial communities. This study emphasizes the importance of microbiome-tumor-drug interactions and highlights the need for future studies in order to advance the development of CRC personalized treatments.

C50	Zinc Deprivation Induces Metabolic Reprogramming and Stress Adaptation in <i>Pseudomonas aeruginosa</i>
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Nutritional immunity, a facet of the innate immune response, limits pathogen growth by modulating micronutrient availability. *Pseudomonas aeruginosa*, a leading cause of mortality in Cystic Fibrosis (CF) patients, colonizes the lung environment where it encounters significant nutrient limitation, particularly of zinc. In fact, low zinc availability has recently emerged as a key factor in controlling lung colonization. Although *P. aeruginosa* employs multiple zinc acquisition systems to counteract host-imposed restriction, the broader metabolic and physiological adaptations that enable its survival under severe zinc deprivation remain poorly defined.

In this study, we analysed transcriptomic changes in wild-type *P. aeruginosa* PA14 and a zinc-uptake deficient mutant (*znuAzrMB*) grown in a chemically defined, zinc-depleted medium (VBMM). RNA-sequencing revealed strong induction of oxidative stress response genes and upregulation of denitrification pathways, suggesting a shift toward anaerobic and microaerophilic respiration under zinc starvation. Additionally, genes associated with central carbon metabolism, motility, quorum sensing, and virulence were found to be significantly downregulated. These transcriptomic changes were validated by RT-qPCR, luminescence-based biosensor assays, and Seahorse analysis. Strikingly, the gene expression patterns observed under zinc starvation mirror the genetic changes commonly observed in *P. aeruginosa* isolates from CF sputum and represent key adaptations for survival in the oxygen- and metal-limited conditions characterizing the mucus environment.

Overall, our findings reveal a novel link between zinc starvation and global metabolic reprogramming, emphasizing zinc availability as a critical environmental cue that thrives adaptive strategies of *P. aeruginosa* in the CF airways.

C51	Antibacterial and antibiofilm activities from marine fungi against <i>P. aeruginosa</i> strains
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Pseudomonas aeruginosa is a common opportunistic Gram-negative pathogen associated with persistent infections, particularly in immunocompromised patients. Its intrinsic and acquired resistance to common antibiotics, due to mechanisms such as efflux pumps and biofilm formation, makes it one of the most difficult pathogens to treat in clinical settings. Therefore, it is necessary to identify new alternative therapeutic strategies. Identifying effective antipseudomonal compounds is crucial not only for improving clinical outcomes but also for tackling broader challenges in areas such as biotechnology.

Neglected poly-extremophilic marine fungi represent a promising source of new bioactive

compounds. Specifically, this study evaluates the antipseudomonal and anti-biofilm activity of extracts obtained from selected strains of marine fungi, including *Penicillium rubens* (HP10), *Clonostachys rosea* (IG119) and a species belonging to the *Phaeosphaeriaceae* family (PN33). Previous analyses have shown that these extracts are active against *P. aeruginosa* ATCC 15442, with MIC values ranging from 0.07 mg/mL to 2.5 mg/mL, depending on the fungal species and extraction solvent. The extracts were also tested against two reference strains, PA01 and PA14. In particular, HP10 and PN33 showed significant antibacterial activity against PA01. As regards the effect on biofilms of tested marine fungi extracts, weak inhibition percentages (less than 50%) were obtained.

Studies are currently ongoing to purify and chemically characterise the active extracts in order to identify compounds that are potentially active against *P. aeruginosa*. Purified bioactive molecules could represent a valid alternative to antibiotics.

C52

Polyamines metabolism and its effects on modulation of macrophage response to *Shigella* invasion: another intriguing brick in the wall

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Polyamines are a class of small polycationic molecules present in all cells. Their fortune in the world of eukaryotes and prokaryotes is due to the multitude of biological functions they perform, including translation, gene regulation, stress resistance, cell proliferation and differentiation, making them essential molecules for life. In bacteria they are clearly emerging as crucial players in pathogenic process.

The question of the importance of polyamines has also been raised in *Shigella flexneri*, the etiologic agent of human bacillary dysentery, where the evolutionary pathway to pathogenicity involved the remodelling of the content and regulation of polyamines to optimize the bacterial fitness in the host. Essentially, *S. flexneri* accumulates spermidine and does not synthesize cadaverine as a result of the inactivation of the *speG* gene, which encodes acetyl spermidine transferase, and *cadA* gene, which encodes lysine decarboxylase, respectively. How does the rearrangement of polyamine metabolism affect the *Shigella* intracellular lifestyle? We tried to address this question by investigating the intramacrophage step of the complex invasion program of *S. flexneri*.

Our results, obtained by infecting THP-1-derived macrophages with *S. flexneri* wild type and derivative strains lacking the main biosynthetic pathways of polyamines, reveal a complex scenario in which putrescine, one of the most common polyamines, may play an intriguing role in bacterial-host interactions. Indeed, quantification of cytokine release from infected macrophages supports the evidence that putrescine export from the bacterium drives *Shigella*'s ability to shape the cell response to its invasion.

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C53	Selective activation of NF-κB by HSV-1 regulates c-Fos and inflammatory responses in monocytic cells
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Herpes Simplex Virus type 1 (HSV-1) employs sophisticated strategies to manipulate host immune responses, enabling long-term persistence. In this study, we investigated the innate immune response to HSV-1 in human monocytic cells, with a particular focus on the MyD88-NF- κ B signaling axis and its regulation. We demonstrated that HSV-1 infection induced TRAF6 (TNF Receptor-Associated Factor 6), leading to NF- κ B activation in a MyD88-dependent manner at the transcriptional level. Indeed, using THP-1-MyD88^{-/-} cells we found the reduction of both TRAF6 and NF- κ B transcripts. Given that NF- κ B is a key factor tightly regulated by multiple signaling pathways and is responsible for controlling the expression of numerous genes, we analyzed NF- κ B downstream target genes in THP-1 wild type cells by RT2 profiler PCR array. HSV-1 infection strongly upregulated chemokines and inflammatory cytokines, a response strictly dependent on viral replication, as shown by phosphonoacetic acid (PAA) treatment. Chemokines reduction was observed in THP-dnkB α , demonstrating that their induction depends on HSV-1-induced NF- κ B activation. We also found that NF- κ B, triggered by HSV-1 replication, contributes to c-Fos activation at transcriptional and translational levels. Our findings support a model in which HSV-1 orchestrates a selective activation of host signaling pathways modulating key regulators to suppress effective viral replication.

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C54	How are gut microbes transmitted? An integrative approach to quantify the ecological factors driving microbiome transfer in guppies
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Microorganisms play an essential role in the health, growth and development of animals, collectively forming the microbiota. Despite their renowned importance, the ecological factors that drive microbiome transfer - such as host contact and dispersal - remain poorly understood. Fish, which comprise the majority of vertebrate species, offer unique advantages for studying microbiome ecology, particularly due to their suitability for controlled experiments and their relevance in aquaculture and conservation contexts. In this study, we chose to investigate the transmission dynamics of the gut microbiome in the guppy (*Poecilia reticulata*). Guppies have been extensively studied in behavioural and ecological research, thus providing a tractable system for accurate measurement of animal contact. Specifically, we aimed to quantify the relative contributions of host contact and dispersal through water to microbiome transfer across individuals. To achieve this, we generated microbiome-depleted guppies, co-housed them in tanks with conventional animals with and without physical contact, and used a multi-faceted experimental approach. This included: (1) identification and quantification of host-host interactions (e.g. contact, mating, nipping) (2) bacterial growth analysis, (3) metagenomic profiling of the fish gut, and (4) fluorescence in situ

hybridization. By integrating these parameters, we identified the predominant transmission routes of microbial populations and elucidated the ecological factors driving the transfer of specific microbial species. This study provides important insights into microbiome dynamics in aquatic environments and highlights the ecological processes underlying microbial transmission in fish, with potential applications for sustainable aquaculture and environmental management.

C55

Levilactobacillus brevis CRAI: a novel GABA-producing probiotic isolated from organic tomatoes

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Gamma-aminobutyric acid (GABA) is one of the most important inhibitory neurotransmitters of the central nervous system and plays a significant role in gut-brain axis. This study explores the ability of lactic acid bacteria (LAB), isolated from different varieties of organic tomatoes, to produce GABA. Thirteen LAB have been isolated, taxonomically identified by 16S rRNA gene sequencing and screened by PCR for the *gadB* (glutamate decarboxylase) gene. The strain positive for *gadB* gene were tested for GABA production, quantified using HPLC. Fermentation was conducted under optimized culture conditions with 4% monosodium glutamate (MSG) and *Levilactobacillus brevis* CRAI showed the best performance in converting MSG into GABA among the isolated LAB. This strain was selected for further characterization: the strain's safety profile was assessed by genome sequencing, that excluded the presence of antibiotic resistance and virulence genes and also revealed the presence of *gadA* and *gadB* gene isoforms. The expression of these genes was evaluated by RT-PCR and turned out to be induced in the presence of 4% MSG. Functional assays were performed to demonstrate the probiotic potential of *L. brevis* CRAI strain. A strong antimicrobial activity was registered against enteropathogens, i.e. *Escherichia coli* ETEC, *Salmonella choleraesuis* and *Yersinia enterocolitica*. In addition, anti-inflammatory effect, in terms of reduction of nitric oxide production in LPS-stimulated RAW264.7 macrophages, was demonstrated. The ability of the strain to adhere to Caco-2 cells was also assessed. These results suggest that *L. brevis* CRAI could be a promising candidate for the development of GABA-enriched nutraceuticals or probiotic supplements.

C56

Phosphatidylinositol 5-phosphate-loaded-apoptotic body-like liposomes for Mycobacterium abscessus infections management in people with cystic fibrosis

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Mycobacterium abscessus (Mab) is an opportunistic nontuberculous mycobacterium, intrinsically resistant to a wide range of antibiotics, responsible of difficult-to-treat pulmonary infections in vulnerable patients, like those with Cystic Fibrosis (CF). Impaired macrophage function in people with CF (pwCF) plays a pivotal role in defective bacterial killing. Despite cystic fibrosis conductance regulator (CFTR) modulators like Kaftrio (ETI) are being widely used to improve

pwCF quality of life and clinical outcome, there still is a proportion of pwCF whose CFTR mutations are incompatible for such pharmacological treatment. In this study, we have investigated a potential therapeutic strategy for managing chronic Mab infections, particularly in pwCF who are ineligible for standard ETI treatments. Results show that both liposome ABL/PI5P and ETI *in vitro* treatments reduce Mab intracellular viability in macrophages from pwCF undergoing or not ETI regimen. Notably, no synergistic, additive or interference effect were shown when the treatments were combined. Furthermore, the *in vitro* stimulation with ABL/PI5P of *in vitro* Mab-infected macrophages from pwCF who did not receive ETI lead to a significant reduction in intracellular mycobacterial viability and the combination of liposomes with amikacin resulted in a further significant reduction compared to single treatments. These findings suggest that ABL/PI5P liposomes, in combination with antibiotics, may represent a valuable therapeutic treatment which can be developed for the control of Mab infections in both patients receiving standard ETI therapy and, most importantly, in pwCF who cannot benefit from such pharmacological treatment.

C57	vB_KpM_GP-7: a novel myovirus targeting capsule-deficient, phage-resistant <i>Klebsiella pneumoniae</i>
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The nosocomial spread of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains represents a significant public health threat, highlighting the urgent need to identify alternative therapeutic strategies. One possible approach is the use of bacteriophages, especially as cocktails made-up by viruses targeting different bacterial receptors. Components of these cocktails can be obtained by using, in phage-screening procedures, strains that have acquired resistance to previously identified phages. Here, we report the identification of vB_KpM_GP-7, a novel lytic phage isolated from urban wastewater using a capsule-deficient, phage-resistant mutant of a clinical MDR *K. pneumoniae* strain belonging to the Sequence Type (ST) 258 clade II. vB_KpM_GP-7 is able to infect a broad spectrum of *in vitro* selected capsule-deficient *K. pneumoniae* strains, including some belonging to STs different from its parental strain. The phage revealed a strictly lytic replication cycle, with a latent period of 25 minutes and an average burst size of 45 particles/infected cell and was stable in a wide pH range (4 – 11) and after incubation at 60° C for an hour. Genomic analysis identified that vB_KpM_GP-7 is a myovirus belonging to the *Marfavirus* genus, with a 169,127 bp linear dsDNA genome in which genes associated with lysogeny or known virulence factors are absent. Frequency of resistance emergence by using high multiplicity of infection was overall low, suggesting a limited risk of rapid resistance development. Altogether, these data highlight that vB_KpM_GP-7 could be a promising candidate for further investigation for its integration into phage cocktails to fight infections caused by MDR *K. pneumoniae* strains.

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C58 Integrating parrotfish microbiome into the coral reef bioerosion paradigm

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Tropical and subtropical coral reefs rank among the most biodiverse marine ecosystems and are major biogenic sources of calcium carbonate (CaCO₃), yet they remain highly sensitive to climate-driven disturbances. Net reef accretion results from a dynamic balance between carbonate production and losses via erosion and dissolution—a balance quantified by the carbonate budget, which serves as an indicator of both reef growth potential and ecological resilience.

Parrotfishes (family Scaridae), particularly excavator species with specialized play a major role in bioerosion and contribute over 80% of new coral-derived sediments, crucial for reef island development. Despite their ecological importance, the role of parrotfish-associated microbiomes in sediment formation remains unknown.

To address this, we analyzed the oral and rectal microbiomes of multiple parrotfish species (including both scrapers and excavators) and compared them with those of the herbivorous powder-blue surgeonfish (*Acanthurus leucosternon*). Using 16S rRNA profiling and shotgun metagenomics, we found that oral and gut microbiomes are shaped by distinct environmental and dietary factors. Parrotfishes, with their high-protein, low-fiber diet, exhibited microbiomes markedly different from those of surgeonfish. Functional analyses revealed dietary specialization among parrotfish groups: excavators harbored microbiomes enriched in protein metabolism, while scrapers showed higher abundance of genes related to fiber and carbohydrate processing. Crucially, metagenomic data also identified microbial genes implicated in both CaCO₃ dissolution and precipitation. We further isolated two bacterial taxa—*Kocuria palustris* and a novel *Rosellomorea* species—capable of directly mediating these processes, suggesting a microbial contribution to coral bioerosion and carbonate sediment production.

C59 Role of anticancer drugs on the emergence of antimicrobial resistance in bacterial pathogens

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Infections caused by multidrug-resistant (MDR) pathogens pose a serious threat to cancer patients, who face a three-fold higher risk of dying from bacterial infections than the general population. While chemotherapy increases infection risk primarily through immunosuppression, certain anticancer drugs also have direct antimicrobial effects. These drugs can disrupt the microbiota and promote the expansion of pathogenic strains. We hypothesised that treatment with anticancer drugs endowed with antimicrobial activity could select for anticancer-resistant

bacterial strains, that in turn could exhibit cross-resistance to antibiotics, further complicating patient outcome.

To investigate this issue, we conducted a screening of anticancer compounds for antimicrobial activity against clinically relevant bacterial pathogens. Several agents demonstrated activity against ESKAPEE pathogens. Notably, spontaneous mutants resistant to selected anticancer drugs exhibited increased resistance to multiple antibiotics compared to their parental strains. Furthermore, we tested a panel of anticancer drugs against MDR and antibiotic-sensitive strains of *Staphylococcus aureus*, an ESKAPEE pathogen whose growth was inhibited by several chemotherapeutics. The MDR strains displayed overall increased resistance to anticancer drugs relative to their sensitive counterparts.

These preliminary findings suggest that chemotherapy may directly contribute to the selection and emergence of MDR bacterial pathogens. Understanding how specific anticancer drugs drive resistance will inform new clinical guidelines for combining chemotherapy and antibiotics, with the goal of mitigating resistance development in cancer patients.

C60

Drug repurposing to inhibit *Pseudomonas aeruginosa* adaptation to the cystic fibrosis lung environment

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Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the lungs of individuals with cystic fibrosis (CF) by exploiting airway mucus (sputum) as a nutrient source. In the CF lung, *P. aeruginosa* forms biofilms that exhibit increased antibiotic tolerance. The set of genes essential for *P. aeruginosa* growth in CF sputum extends beyond those identified in standard laboratory media, and biofilm development is tightly regulated in response to host-specific cues. These findings suggest that previously uncharacterized molecular pathways play a critical role in *P. aeruginosa* persistence and biofilm formation within the CF lung environment. This project aimed to identify new drugs that inhibit these pathways and are thus capable of specifically reducing *P. aeruginosa* growth or biofilm formation under conditions that mimic CF sputum. To this end, a library of over 3,000 FDA-approved compounds was screened in the Synthetic Cystic Fibrosis Sputum Medium (SCFM). Several drugs with previously unrecognized antimicrobial or antibiofilm activity against *P. aeruginosa* were identified. These compounds demonstrated promising *in vitro* efficacy against the reference strain PAO1 and a panel of clinical CF isolates. Ongoing studies are evaluating the activity of these hits in *in vivo* infection models and investigating their mechanisms of action.

Given their favorable pharmacokinetic and safety profiles, these repurposed drugs hold strong potential for clinical application in reducing *P. aeruginosa* burden and antibiotic resistance in the CF lung.

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C61

TYPE I IFNS AFFECT ANTIVIRAL PROTEIN CONTENT OF EVS RELEASED BY HUMAN LUNG EPITHELIAL CELLSZ. Percario, F. Giannessi, A. Sabatini, S. Ciolfi, A. Sacchi, E. Affabris*Roma Tre University, Department of Science, Italy***Background and Aims**

The main antiviral response of the mucosal innate immune system is mediated by type I interferons (IFNs) and recent evidence shows that the extracellular vesicles (EVs) are also involved in the antiviral response. However, data on whether IFNs could affect the information conveyed by EVs are missing. We analyzed if type I IFNs (IFN α 2b and IFN β) influence the production and the characteristics of the released EVs.

Methods

We treated A549 lung carcinoma cells and MRC-5 normal diploid lung embryo fibroblast cells with IFN α 2b and IFN β . Then, we purified large-EVs (IEVs) and small-EVs (sEVs) through serial ultracentrifugation steps. The number and dimension of collected sEVs were evaluated by Nanoparticle Tracking Analysis, and the presence of some IFN-induced antiviral proteins was analyzed by western blot.

Results

Hu-IFN β and hu-IFN α 2b markedly increase the presence of the analysed protein (IFITM1, IFITM3, BST-2, APOBEC3G and ISG15) inside the MRC-5 cells. All these proteins were present in the small microvesicle (small EV) harvested from IFNs-treated MRC-5 cells except ISG15 that was barely detectable. BST-2 and APOBEC3G are not detected in small EV released by A549 cancer cell line.

Conclusions

Our results show that the type I IFNs influence the content of IFN-induced proteins in EV from MRC-5 normal embryonic lung cells, but EV released by A549 lung cancer cells do not show the same phenotype, possibly modifying their influence on target cells.

C62

Probiotics and Immune Response in *C. elegans*: Effects of Milmed yeast against Human PathogensE. Schifano, L. Pompa, A. Scornajenghi, D. Uccelletti*Department of Biology and Biotechnologies Charles Darwin, Sapienza University of Rome, Italy*

Caenorhabditis elegans is a prominent model for studying host–pathogen interactions due to its simple anatomy, conserved molecular pathways, and well-mapped neural network. Inhabiting soil and decaying fruits, it encounters various bacteria, including pathogens. Despite lacking specialized immune cells and adaptive immunity, *C. elegans* has evolved effective innate defenses, including antimicrobial responses in the intestine, hypodermis, and nervous system. It also exhibits neural learning to recognize and avoid pathogens. Key immune pathways, such as p38 MAPK, TGF β , bZIP transcription factors, and nuclear hormone receptors, are conserved with mammals, facilitating insights into innate immunity.

C. elegans has also been employed to investigate the potential anti-inflammatory effects of various probiotics, primarily attributed to their antioxidant, anti-aging, and lifespan-extending properties, as well as their capacity to enhance the nematode's immune response by increasing resistance to bacterial pathogen infections.

Herein we report that the probiotic Milmed yeast, obtained from *S. cerevisiae* after exposure

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to electromagnetic millimeter wavelengths, induces a protection in *C. elegans* infected animals with different human pathogens. The investigation of the innate immunity pathways involved in the interaction between yeast and nematode has been performed through genetic and cellular approaches, taking advantages of mutant and transgenic strains.

C63

Thiostrepton interferes with *Pseudomonas aeruginosa* quorum sensing systems inducing autolysis and altering the production of virulence factors

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Pseudomonas aeruginosa is a Gram-negative bacterium known to be an opportunistic pathogen that causes life-threatening infections affecting mainly immunocompromised patients and those with cystic fibrosis. It is resistant to many antibiotics and produces a variety of virulence factors, like pyocyanin and pyoverdine, that help it adapt and survive in different environments. Thiostrepton is a thiopeptide antibiotic that inhibits the stringent response (SR), a mechanism that allows the survival of bacteria in stressful conditions regulating also the expression of virulence factors. We found that growing *P. aeruginosa* strain PAO1 on solid medium containing a low dose of thiostrepton induces an autolytic process probably related to quorum sensing (QS), as it occurs where bacteria have a higher cell density, normally characterized by pigmentation. Growing in the same conditions an environmental isolate of *P. aeruginosa* highly producing pyocyanin, seen as intense green-blue pigmentation, we observed a similar phenotype with total absence of pigmentation. Interestingly growing PAO1 in liquid medium with the same dose of thiostrepton has shown little effect on bacterial growth but a high increase in the production of pyocyanin and a decrease in that of pyoverdine. These data, although apparently contrasting, highlight the ability of thiostrepton to alter the virulence of *P. aeruginosa*, probably acting on the interplay between the SR and the intricate QS systems of this bacterium. Furthermore, it emerges a possible different effect of this molecule depending on growth conditions aligning with the high versatility shown by this bacterium.

C64

Exploring Natural Products for Host-Directed Therapies Against *Mycobacterium abscessus*

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Mycobacterium abscessus is a rapidly growing non-tuberculous mycobacterium (NTM) increasingly recognized for its role in chronic pulmonary infections, particularly in individuals with underlying lung conditions such as cystic fibrosis. Its intrinsic resistance to a wide range of antibiotics poses significant therapeutic challenges and highlights the urgent need for alternative treatment strategies.

Recent research has begun to explore host-directed therapies (HDTs), which aim to modulate the host immune response rather than targeting the pathogen directly. This approach offers the potential to enhance bacterial clearance while limiting tissue damage. In parallel, natural products

have emerged as a rich source of bioactive compounds with immunomodulatory and antimicrobial properties.

Our current project investigates active compounds isolated from natural products, collected from central and coastal regions in Brazil known for their exceptional chemical biodiversity, as well as semi-synthetic scaffolds designed on these natural products. Preliminary data from related studies on *Mycobacterium tuberculosis* suggest that several classes of compounds, including chalcones, thioureas, and flavones, may hold promise for anti-mycobacterial activity and immune modulation.

As an ongoing international collaboration, this work aims to assess the potential of these natural products as novel therapeutic candidates against *M. abscessus*, with a focus on identifying those that could function as HDTs, and contributes to the growing field of alternative antimycobacterial strategies, seeking to expand the therapeutic toolkit against a pathogen of rising clinical concern.

C65

Investigating the Role of the AraC/XylS Family Transcriptional Regulator VirF in *Acinetobacter baumannii* Virulence

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Acinetobacter baumannii is a major cause of nosocomial infections and a growing public health threat due to its resistance to multiple antibiotics. As antibiotic options diminish, targeting virulence factors that drive pathogenesis presents a promising alternative therapeutic approach. *A. baumannii* utilizes a diverse array of virulence factors, yet many pathogenesis regulatory mechanisms remain poorly understood. Identifying new regulators can unveil novel therapeutic targets. AraC/XylS family transcriptional regulators (AFTRs) play key roles in controlling virulence, stress responses, and metabolism in pathogens such as *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. We identified a putative AFTR in *A. baumannii*, designated *virF*, and hypothesize it may function as a virulence regulator. Structural modeling revealed conserved AFTR features, including a DNA-binding domain and a jelly roll-folded companion domain, supporting a potential regulatory role. To assess its relevance, we performed a conservation analysis of *virF* across a broad panel of clinical isolates. Strikingly, *virF* was found to be highly conserved, despite the well-documented genomic plasticity of *A. baumannii*. This high level of conservation across diverse clinical strains strongly suggests a crucial role in the bacterium's fitness or pathogenicity, and highlights *virF* as a clinically relevant target for anti-virulence strategies. To explore its function, we are generating *virF* deletion mutants using a markerless, double-homologous recombination method with counter-selection. Ongoing studies aim to assess the effect of *virF* disruption on virulence-associated phenotypes and host-pathogen interactions. Our findings may uncover a conserved, druggable regulator essential to *A. baumannii* virulence, with potential implications for novel therapeutic development.

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C66	Engineering a Dual-Fluorescent HSV-1 Using <i>En Passant</i> Mutagenesis: A Tool for Herpesvirus Replication Studies
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HSV-1 causes one of the most widespread viral infections in the human population. A powerful method for studying the mechanisms of viral infection uses genetically encoded fluorescent fusion tags to label proteins.

This study aims to construct a recombinant HSV-1 that encodes two selected structural proteins, the capsid protein VP26, fused with the monomeric green fluorescent protein mNeon, and the envelope glycoprotein gH, fused with the monomeric red fluorescent protein mScarlet, via BAC mutagenesis technique, to study various aspects of the herpesvirus life cycle. BACs are single-copy plasmids derived from the *E. coli* F-factor, able to carry the HSV genome as an infectious clone. *En passant* mutagenesis, termed “two-step markerless Red recombination system”, consists of two recombination steps: a selectable PCR product containing the desired sequence is inserted into the appropriate locus in the BAC, then the selectable marker is excised, producing a scarless mutation in the gene of interest. To produce recombinant BAC expressing the fluorescent proteins, BAC DNA was transfected into permissive Vero cells and positive clones were recovered to produce new viral stock. The constructed recombinant virus was sequenced by Sanger and deep sequencing, revealing no frameshift mutations, and characterized in Vero cells by plaque assay, western blotting, and qPCR techniques, confirming wild-type-like behavior. The double recombinant HSV-1 was then used to infect permissive and semi-permissive cell systems, lacking caspase-8, with the aim of studying the virus-host interaction, and gaining insight into the role of caspase-8 in HSV-1 infection.

C67	cIRO: a novel cell-based platform to investigate SARS-CoV-2 replication organelles formation
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During SARS-CoV-2 replication, the host cell undergoes a profound reshaping at a structural and metabolic level. In particular, the ER membranes are hijacked and remodelled by two key players, NSP3 and NSP4, viral non-structural proteins that suffice to generate viral replication organelles (vROs), tailored compartments in which viral genome replication occurs (Cortese, 2020). Since vROs are evolutionarily conserved structures among several +ssRNA viruses, targeting their biogenesis could be an effective strategy to identify key host pathways implicated in +ssRNA viral replication, which are still largely elusive.

In this perspective, we developed a robust inducible cell system, called cIRO (cellular-Inducible viral Replication Organelles platform) in which NSP3 and NSP4 were stably introduced using lentiviral delivery. The reporter cell line allows the monitoring of DMVs clustering by high-content imaging, thus being suitable for high-throughput applications. We screened a library of 160 compounds active on cellular metabolism, and we were able to identify changes in the NSP3-

NSP4 pattern using an AI-based approach for image analysis.

Interestingly, our screening revealed two key pathways implicated in DMVs biogenesis: autophagy and lipid metabolism. These cellular routes are known to be hijacked upon infection by +ssRNA viruses, although the precise replication steps in which they are required are still largely unknown. We confirmed in a fully infectious SARS-CoV-2 context that these pathways are implicated in the early establishment of viral replication, thus proving the reliability of our cIRO platform and the importance of key host factors in the autophagic flux for DMVs formation.

C68

Comprehensive characterization of a novel dispirotriperazine-based antibiotic adjuvant against *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is the most prevalent cystic fibrosis pathogen, causing severe lung infections that are extremely hard to eradicate, due to the fast development of multidrug-resistance. This leads to the decline of antibiotics efficacy and highlights the need for novel antibiotic adjuvants (AA) to restore their effectiveness. PDSTP is a dispirotriperazine antiviral compound that, in our laboratory, has been repurposed as AA against *P. aeruginosa*, but its spectrum of activity, safety and mechanism of action (MoA) remain elusive. To elucidate these points, first, PDSTP AA potential was investigated by checkerboard assays, showing a strong synergy in combination with ceftazidime, rifampicin, nalidixic acid and colistin against a panel of *P. aeruginosa* CF clinical isolates. Then, using MTT assays, its highly safe profile towards different human lung epithelial cell lines was proved. Concerning the MoA, PDSTP displayed high affinity for *P. aeruginosa* lipopolysaccharide (LPS) and the ability to impair outer membrane integrity. These data were obtained by dansyl-polymyxin B displacement assays and scanning electron microscopy, respectively. Finally, combining nitrocefin degradation, propidium iodide internalization and ATP release assays, PDSTP effect towards outer and inner membrane was determined against both PAO1 wild-type and a laboratory-evolved colistin-resistant PAO1 strain (PAO1 ColR1) possessing aminoarabinosylated lipid A. Overall, this work has significantly improved our knowledge of PDSTP as AA, showing a broad spectrum of activity, no cytotoxicity and unveiling its peculiar MoA.

This work was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

C69

Synthetic STING-targeting compounds as potential novel antiviral agents against HSV-1

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Herpes Simplex Virus type 1 (HSV-1) is a widespread human pathogen capable of evading the host's innate immune response, primarily by disrupting the type I interferon signaling cascade. A central player in the antiviral innate immune system is the STING (Stimulator of Interferon Genes) pathway, which detects cytosolic viral DNA and activates the production of type I interferons, along with pro-inflammatory cytokines. HSV-1 can interfere with this pathway, diminishing the host's capacity to mount an effective immune response. In light of this, pharmacological activation of STING has gained interest as a potential strategy to restore innate immunity and counteract viral immune evasion. Synthetic STING agonists have shown promising antiviral activity in preclinical models. This study aimed to identify new molecules capable of activating the STING pathway and inhibiting HSV-1 replication. A library of 52 rationally designed synthetic compounds was screened in silico for predicted binding affinity to the human STING substrate pocket using molecular docking. Based on scoring and chemical diversity, two representative compounds were selected for experimental validation. Their antiviral efficacy was assessed using a plaque assay in Vero cells, and STING pathway activation was evaluated by measuring STING phosphorylation levels via Western blot analysis.

Both compounds demonstrated antiviral activity by reducing the release of HSV-1 particles and viral protein expression. Notably, only one compound induced STING phosphorylation, indicating a STING-dependent mechanism, while the other likely acted through a STING-independent pathway. These findings highlight the potential of both STING-targeting and STING-independent molecules as novel antiviral candidates against HSV-1.

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C70	Genotype-by-genotype interkingdom interactions: molecular insights into the cross-talk between <i>Sinorhizobium meliloti</i> and <i>Trichoderma</i> species for rhizomicrobiota assembly
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Microbial interactions are key drivers of microbiota functionality and ecosystem dynamics, profoundly influencing plant health and productivity. In the rhizosphere, *Trichoderma* species and rhizobia are of particular importance due to their well-established roles in promoting plant growth and providing protection against pathogens.

In this work, we present an integrated analysis of the interactions between the symbiotic nitrogen-fixing bacterium *Sinorhizobium meliloti* and soil fungi of the genus *Trichoderma*, with the aim to test the hypothesis of a genotype × genotype interaction between them, shedding light on the molecular determinants underlying these interactions. We seek to provide insights into their potential impact on plant-microbe symbioses, as well as those factors shaping the success of microbial consortia, for more sustainable agricultural practices.

We combined microbiological observation, physiological, metabolic, and transcriptomic analyses, to investigate the effects of fungal spent media from four *Trichoderma* species (*T. velutinum*, *T. tomentosum*, *T. gamsii*, and *T. harzianum*) on four strains of *S. meliloti*. We then focused on the strain-specific molecular recognition by transcriptomic analyses of *T. velutinum* in presence of different *S. meliloti*.

Results revealed strain- and species-specific effects on rhizobial physiology and transcriptomics, with up to 25% of rhizobial genes differentially expressed. These changes significantly influenced the symbiotic performance of *S. meliloti* with *Medicago sativa*, demonstrating complex genotype × genotype interactions between fungi and bacteria. On the fungal side, the transcriptome varied with bacterial strain. Strikingly, ~90% of these genes lacked functional annotation, pointing to an uncharacterized genetic space critical for fungal-bacterial communication.

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