SIMGBM
Società Italiana di
Microbiologia Generale
e Biotecnologie Microbiche

Microbiology 2023

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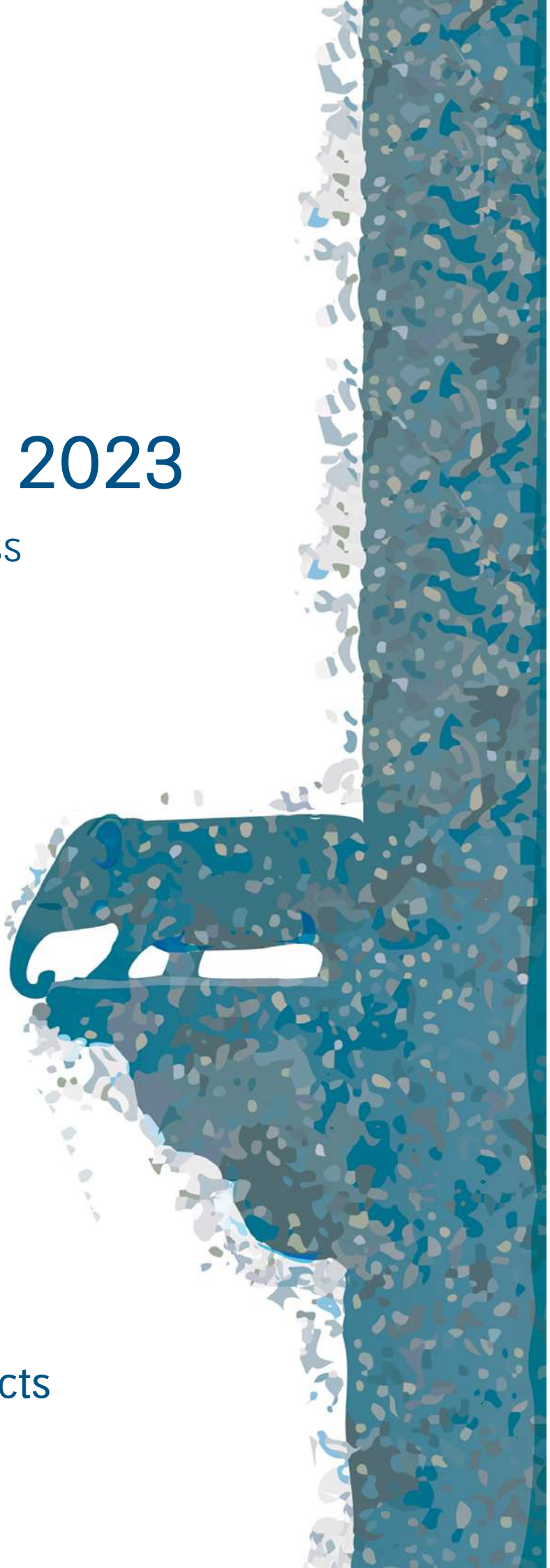
Cagliari

September 21-24, 2023



Programme and abstracts

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Programme

Thursday, September 21st

17.30-19.30 Registration

18.30-19.30 | OPENING LECTURE

Chairs: **P. Landini, G. Rampioni**

Natalia Tschowri (*Leibniz University, Hannover, Germany*)
Cyclic-di-GMP signalling in bacterial differentiation and metabolism

19.30 Welcome Cocktail at the Botanical Garden

Friday, September 22th

From 8.00 Registration

09.00-11.00 | PLENARY SESSION 1
Biology of nitrogen-fixing bacteria and their applications

Chairs: **M. Bazzicalupo, M. Varcamonti**

09.00 **Alessio Mengoni** (*Università di Firenze*)
Evolutionary genomics of nitrogen fixing bacteria

09.45 **Marco Nuti** (*S.S. Sant'Anna, Pisa*)
Application of nitrogen fixing bacteria in sustainable agriculture

10.10 **Carmen Bianco** (*IBBR-CNR, Napoli*)
Bacterial production of indole-3-acetic acid (IAA) and its effect on nitrogen fixation in different host plants

10.35 **Maurizio Chiurazzi** (*IBBR-CNR, Napoli*)
Genetics of nitrate transport and signaling in N-fixing symbioses

11.00-11.30 Coffee break

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11.30-13.30 | PLENARY SESSION 2

Metabolic adaptation to stress conditions

Chairs: **L. Baccigalupi, F. Imperi**

11.30 **Forest Rohwer** (*SDSU, San Diego, CA, USA*)
The holobiont approach in health and disease

12.15 **Pietro Alifano** (*Università del Salento, Lecce*)
Metabolic changes of *Neisseria meningitidis* during host infection

12.40 **Gianni Prosseda** (*Università Sapienza Roma*)
Polyamine metabolism during *Shigella* infection

13.05 **Emanuela Frangipani** (*Università di Urbino*)
Fighting *Staphylococcus aureus* through iron starvation

13.30-15.30 Lunch & Posters viewing (odd numbers)

15.30-16.30 | **PLENARY LECTURE 1**

Chairs: **D. Cavalieri, E. Ricca**

Silvia Bulgheresi (*University of Vienna, Austria*)
Cell biology of bacteria that live on the surface of animals

FRIDAY

16.30-16.45 | **SHORT PRESENTATION**

Margherita Sosio (*NAICONS, Milano*)
Presentation of the micro4all Platform, the first “Search-and-Order Molecules Engine”

16.45-17.30 | **PRIZE PRESENTATION**

FRANCO TATÒ Prize 2023

Annapaola Petrosino (*Università di Bologna*)
Nanobiotechnological engineering of the M13 phage into an orthogonal platform for biomedical applications

GENPROBIO Prize 2023

Stefany Castaldi (*Università di Napoli*)
Biology and application of industrial microorganisms

SIMGBM Prize 2023 (Kindly supported by Microorganisms)

Angelica Pellegrini (*Università di Pavia*)
The global transcriptional regulator CodY controls virulence in Group B *Streptococcus*

17.30-19.00 | **ANNUAL ASSEMBLY OF SIMGBM MEMBERS**

20.30 Social dinner

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Saturday, September 23rd

09.00-11.00 | PLENARY SESSION 3

Cell envelope remodeling to shape bacterial fitness in response to the host and environment

Chairs: **B. Colonna, A. Polissi**

09.00 **Simonetta Gribaldo** (*Institut Pasteur, Paris, France*)
One or two membranes? Illuminating the evolution of the cell envelope across the Tree of Bacteria

09.45 **Víctor Hernández-Rocamora** (*Newcastle University, UK*)
Peptidoglycan remodeling and bacterial fitness under stressful conditions

10.20 **Alessandra Martorana** (*Università di Milano*)
Peptidoglycan remodeling and amidases activation under lipopolysaccharide biogenesis defects

10.40 **Orietta Massidda** (*Università di Trento*)
Regulation of peptidoglycan synthesis during growth and division

11.00-11.30 Coffee break

11.30-13.30 | PLENARY SESSION 4

Functional microbial diversity in polluted environments

Chairs: **A. Franzetti, M. Petruccioli**

11.30 **Ibrahim M. Banat** (*University of Ulster, Northern Ireland, UK*)
Microbial biosurfactants: current trends and applications in environmental, agricultural and biomedical industries

12.15 **Isabella Gandolfi** (*Università di Milano-Bicocca*)
Microbial ecosystem functions in polluted environments

12.40 **Silvia Crognale** (*Università della Tuscia, Viterbo*)
Mycoremediation in TPH polluted environments: fungal and bacterial interactions

13.05 **Elena Tamburini** (*Università di Cagliari*)
Benthic microbial response to pollutants in anthropized marine sediments

13.30-15.30 Lunch & Posters viewing (even numbers)

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15.30-16.30 | PLENARY LECTURE 2

Chairs: **P. Visca**

Ray J. Turner (*University of Calgary, Alberta, Canada*)
Role of metal-based antimicrobials in the Antimicrobial Resistance Era

16.30-19.00 | PARALLEL SESSIONS: short talks from selected abstracts

SESSION A: MICROBIAL GENETICS AND GENOMICS
Chairs: **M. Ventura, E. Rossi**

Carmen Apicella *(Università di Siena)*
Prophage Φ1207.3 is responsible for a temporary activation of a mutator phenotype in *Streptococcus pneumoniae* upon irradiation at different UV-C light wavelengths

Valerio Baldelli *(Università di Milano)*
Functional characterization of the RS04555 gene and its association with persistent cystic fibrosis lung infections of *Pseudomonas aeruginosa*

Claudia Campobasso *(Università di Pisa)*
Antibiofilm activity of a *Staphylococcus aureus* phage: potential key role of its baseplate protein

Giulia Degiacomi *(Università di Pavia)*
A new drug candidate against *Mycobacterium abscessus* and other cystic fibrosis pathogens

Andrea Firrincieli *(Università della Tuscia)*
Structural and functional analysis of the active cow rumen's microbial community provides a catalogue of genes and microbes participating in the deconstruction of cardoon biomass

Gabriele Andrea Lugli *(Università di Parma)*
The infant gut microbiota atlas: detailed insights from composition to functional microbe-based biodiversity

Marta Mellini *(Università Roma Tre)*
RsaL-driven negative regulation promotes heterogeneity in *Pseudomonas aeruginosa* quorum sensing

Valentina Pastore *(Università Sapienza Roma)*
Improved antimicrobial activity of colistin in combination with putative ArnT inhibitors in *Pseudomonas aeruginosa* biofilms

Anella Saggese (*Università di Napoli)*
Charcaterization of carbohydrate active genes carried by an intestinal isolate of *Bacillus subtilis*

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Davide Sposato *(Università Roma Tre)*
AsmA-like proteins are essential but redundant for growth and cell envelope integrity in *Pseudomonas aeruginosa*

Ivana Staiano (*Università di* Napoli)
Critical role of CotE in spore coat proteins deposition at different temperatures of growth

Makrina Totsika *(QUT, Brisbane, Australia)*
O antigen production sensitises *Escherichia coli* to bile salts – A plausible explanation for how *E. coli* K-12 lost its O antigen

SESSION B: ENVIRONMENTAL AND INDUSTRIAL MICROBIOLOGY
Chairs: **P. Quatrini, C.E. Parolin**

Angelina Cordone *(Università di Napoli)*
The Southern Ocean microbial community structure: a response to climate change

Lapo Doni *(Università di Genova)*
Long-distance dispersals govern the global biogeography of Vibrio spp. in the ocean

Sara Del Duca *(CREA, Firenze)*
Towards a harmonized system for the monitoring of soil microbial biodiversity

Stefania Di Silvestro *(Università di Bologna)*
Study of a tellurite hyper-resistant mutant to delve into *Rhodococcus* stress response and resistance mechanisms to toxic metalloids

Sara Borin *(Università di Milano)*
Rhizoremediation potential in a historical polychlorinated biphenyl polluted site

Melinda Mandaresu *(Università di Cagliari)*
Bioaugmentation-assisted phytostabilization of Sardinian abandoned mines by plant-growth promoter Serratia sp.

Roberto Mazzoli (*Università di Torino)*
Prebiotics, antibiotics and platform chemicals from enological by-products: a sustainable bio-economy perspective

Immacolata Serra *(Università di Milano-Bicocca)*
Engineered *Saccharomyces cerevisiae* for the upcycling of polyethylene terephthalate (PET) monomers

Ylenia Di Leto *(Università di Palermo)*
Modulation of sewage sludge microbiome for enhancing the transition from wastewater treatment plants into biorefineries in the circular economy era

Letizia Fracchia *(Università Piemonte Orientale)*
Bacillus subtilis AC7 Fermentation on Rice Husk Substrate: A Sustainable Approach for Lipopeptide Biosurfactant Production

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Annamaria Bevivino *(ENEA, C.R. CASACCIA, Roma)*
Unlocking the potential of microbiome-based solutions as green biofertilizer for sustainable agricolture

Giulia Alessandri *(Università di Parma)*
Assessment of the species-level infant gut bacterial biodiversity through a meta-analysis and the formulation of an optimized cultivation medium

SESSION C: INTERACTIONS BETWEEN MICROBES/VIRUSES AND THEIR HOSTS
Chairs: **A. Tavanti, N. Grandi**

Elisabetta Affabris *(Università Roma Tre)*
The influence of interferons on extracellular vesicles produced by primary monocyte derived macrophages

Mirko Cortese *(Università Campania, Caserta)*
Organelle remodeling by positive-sense single-stranded RNA viruses

Chiara Tarracchini *(Università di Parma)*
Microbial genetic strategies for sex-specific gut persistence throughout the human life

Maria Vittoria *(Università di Napoli)*
Bacillus spores exert a protective and immunomodulatory activity in mice with DSS-induced colitis

Davide Sorze *(Università di Padova)*
LysX2 is a *Mycobacterium tuberculosis* membrane protein with an extracytoplasmic MprF-like domain

Serena Ammendola *(Università Tor Vergata, Roma)*
The Zn/Cd efflux systems of *Salmonella* Typhimurium are critical for the colonization of plants

Stefano Nenciarini (*Università di Firenze)*
Characterisation and immunomodulatory potential of extracellular vesicles from non-pathogenic yeast strains isolated from a fermented product

Sabrina Tamburini *(Università di Venezia)*
Engraftment of Viable Microbiota after Fecal Transplantation Drives *Clostridiodes difficile* Inhibition

Marco Coluccia *(Università Sapienza, Roma)*
Role of the MDR Efflux Pump AcrAB in Epithelial Cell Invasion by Shigella flexneri

Annalaura Paulis *(Università di Cagliari)*
Identification of new benzofuran derivatives as STING agonists with broad-spectrum antiviral activity

Luca Ulfo *(Università di Bologna)*
Genetically Modified M13 Bacteriophages for Precise Photodynamic Cancer Eradication

Dimitrios Vagenas *(QUT, Kelvin Grove, Australia)*
Fitting the best statistical model: A case study on longitudinal urinalysis data of uropathogenic *Escherichia coli* infection in mice.

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Sunday, September 24th

JOINT MEETING

Società Italiana di Microbiologia - **SIM**

Società Italiana di Microbiologia generale e Biotecnologie Microbiche - **SIMGBM**

Sessions held at Palazzo Doglio

09.00 Greetings from the Societies' Presidents

9.30-13.00 | PLENARY SESSION 5

Novel therapeutic approaches against viruses

Chairs: **M. Galdiero, M. Pistello, E. Tramontano**

9.30 **Benjamin Berkhout** (*University Medical Centers, Amsterdam, NL*)
CRISPR-Cas gene editing approaches to attack the HIV provirus in the cellular reservoir

10.10 **Raffaele De Francesco** (*Università di Milano*)
New therapies on the horizon for hepatitis B: are we close to a cure?

10.50-11.20 Session break

11.20 **Angela Corona** (*Università di Cagliari*)
Exploiting Ebola virus suppression of the innate immune activation as target for drug development

12.00 **Rob Lavigne** (*University of Leuven, Belgium*)
Phage genomics and therapy

13.00-14.00 Lunchtime break

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14.00-17.00 | PLENARY SESSION 6

Bacterial physiology: back to the future of Microbiology

Chairs: **P. Landini, G. Pozzi, M. Ventura**

14.00 **Frédéric Barras** (*Institut Pasteur, Paris, France*)
Redox condition adaptation, energy metabolism and antibiotic sensitivity in *Escherichia coli*

14.40 **Paolo Visca** (*Università Roma Tre*)
Bacterial iron metabolism; turning basic physiology into drug development

15.20 **Giovanni Delogu** (*Università Cattolica, Roma*)
At the crossroad of metabolism and bacterial pathogenesis: the case of PE and PPE proteins of *M. tuberculosis*

16.00 **Francesco Santoro** (*Università di Siena*)
Analysis of host-pathogen interaction by dual RNA sequencing: methods and applications

16.40-17.00 Round table discussion

ABSTRACTS

OPENING LECTURE

Cyclic-di-GMP signalling in bacterial differentiation and metabolism

N. Tschowri

Leibniz Universität Hannover

Streptomyces are our most prolific antibiotic producers and represent an excellent system to study multicellular differentiation. They live in soil, where they encounter diverse environmental cues that trigger antibiotic production and a complex transition from multicellular filaments to spores. The widespread bacterial second messenger bis-3´-5´-cyclic di-guanosine monophosphate (c-di-GMP) is a key regulator of the hypha-to-spore transition and antibiotic biosynthesis in *Streptomyces*. C-di-GMP signals are integrated into the genetic network controlling cell differentiation by the regulator BldD and the sigma factor WhiG. Our analysis of c-di-GMP-specific diguanylate cyclases and phosphodiesterases revealed that c-di-GMP determines timing and mode of sporulation by affecting genes involved in cell division and the production of the hydrophobic sheath that covers *Streptomyces* aerial hyphae and spores. In a screen for novel c-di-GMP-effectors, we identified the glycogen debranching enzyme GlgX as a new class of c-di-GMP-binding proteins. Using biochemical and structural analysis we showed that c-di-GMP binding stimulates GlgX-mediated glycogen breakdown by stabilizing the active conformation of the enzyme. Our structures of apo GlgX and the GlgX-c-di-GMP complex revealed that c-di-GMP induces long-range structural changes leading to the reorganization of the GlgX catalytic pocket. A strain with decreased glycogen levels is compromised in sporulation, suggesting that glycogen is needed for cell differentiation. Overall, we identified a new function of the second messenger c-di-GMP to control energy storage metabolism in bacteria.

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PLENARY LECTURES

Cell biology of bacteria that live on the surface of animals

T. Viehboeck[1], P. Weber[1], N. Krause[1], V. Lioy[2], I. Junier[3], **S. Bulgheresi**[1]

[1]*Department of Functional and Evolutionary Ecology, University of Vienna, Austria*

[2]*Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France*

[3]*TIMC, Institut Jean Roget, Université Grenoble Alpes, La Tronche, France*

Our planet may host up to a trillion prokaryotic species belonging to over 1,000 different phyla. However, we are studying the molecular cell biology of only a dozen of them. This gravely limits our understanding of key life processes such as cell growth and division. Here, I will present what we have learned from studying non-model Gammaproteobacteria that are exclusively found on the surface of worms or humans. The former are *Candidatus* Thiosymbion bacteria that thrive on the cuticle of marine nematodes. The latter are multicellular *Neisseriaceae* that inhabit the mouth of one out of two humans. After presenting their extraordinary cell division modes and discussing the configuration and conformation of their chromosomes, I will conclude with some evolutionary considerations.

Role of metal-based antimicrobials in the Antimicrobial Resistance Era

R.J. Turner
Department of Biological Sciences, University of Calgary, Canada

Antimicrobial resistance (AMR) continues to evolve into one of the most significant public health threats facing the world today. The use of metal ions as antiseptics has been around since antiquity, now, under AMR, metal/metalloid-based antimicrobials (MBA) are being rediscovered and show promise for sustainable control of infectious diseases with a few established for antimicrobial treatment, particularly silver and copper. Regardless, their mechanism of action is poorly understood with misconceptions abound in the literature, and besides specific metal resistance determinants, there is even less understood about metal tolerance with even less considerations for differences between planktonic and biofilm states. Yet a number of other metal(loid)s and various types of formulations (including nanomaterials) are being increasingly considered as antibiotic replacements. My group has utilized a number of microbial, biochemical, and 'omic approaches to obtained information of the system view of bacteria under metal(loid) stress. The data demonstrates that different bacteria respond differently to different metals. Additionally, the breadth of physiological response and targeted biochemical processes is more extensive than previously considered, defining genes and systems not previously identified to be involved in metal sensitivity or tolerance. Not surprisingly, we have noted that growth conditions, physiological state, and metal speciation/formulation can lead to surprising differences in the bacterial response towards MBA's. Here, outcomes from a number of different research questions will be presented. Additionally, our state of developing novel metal mixture antimicrobial formulations targeting various bacteria and applications will be briefly overviewed.

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Plenary Session 1
Biology of nitrogen-fixing bacteria and their applications

Evolutionary genomics of nitrogen fixing bacteria

A. Mengoni
Department of Biology, University of Florence, Italy
E-mail: alessio.mengoni@unifi.it

Many molecular signals are exchanged between rhizobia and the host legume plant; some of them are crucial for the symbiosis to take place, while others are modifiers of the interaction. These latter can have great importance in competition with the soil microbiota and in genotype-specific perception of host plant[1].

Data on strain-specific and host genotype-specific interaction between rhizobia and legumes are shedding light on the genetic determinants of such modifiers, pointing to the relevance of transcriptional variation and the dispensable genome pool[2]. More recent findings are also showing the presence of rhizobial strain-specific interaction with fungi at the level of the rhizosphere microbiota. Finally, strain-by-strain epigenomic variation is emerging as a novel factor of rhizobial genomic variability, which could influence phenotypic plasticity, host plant symbiotic interaction and strain differentiation. Here, we will discuss results emerging from such studies from the perspective of evolutionary interpreting the existence of the high genomic variation in the symbiotic rhizobium *Sinorhizobium meliloti* and its exploitation in the tailored design of synthetic communities[3].

References

[1]Batstone RT, et al. (2022) Genome-wide association studies across environmental and genetic contexts reveal complex genetic architecture of symbiotic extended phenotypes. *mBio*. 13(6):e01823-22.

[2]Fagorzi C, et al. (2021) Non-additive transcriptomic signatures of genotype x genotype interactions during the initiation of plant-rhizobium symbiosis. *mSystems* 6: e00974-20

[3]Fagorzi C, et al. (2023) When biodiversity preservation meets biotechnology: the challenge of developing synthetic microbiota for resilient sustainable crop production. *J Sustain Agric Environ*. 2(1): 5-15

Application of nitrogen fixing bacteria in sustainable agriculture

M. Nuti[1,2], L. Ercoli[1], E. Pellegrino[1]

[1]Sant'Anna School of Advanced Studies, Pisa, Italy

[2]University of Pisa, D:A:F:E., Italy

The dinitrogen fixing microbes are known since 1888, and their first application dates back to 1896, when a patent was filed for the inoculation of legumes with pure cultures of (dinitrogen-fixing) bacteria. 135 years later the following dinitrogen-fixing groups are described in the literature: (1) The so-called free-living heterotrophs, present in the soil/water phase, able to fix a few kg of N_2/ha, the most representative of which include Azotobacter, Beijerinckia, Derxia, Klebsiella, Clostridium, Desulfovibrio, and Desulfotomaculum; (2) Free-living phototrophs, present mainly in the water phase; (3) The symbiotic phototrophs of Gymnosperms, lichens, mosses, ferns; (4) The symbiotic/biocoenotic prokaryotes of Gramineae (*Poaceae*), Leguminosae (*Fabaceae*), *Ulmaceae*, and tree Angiosperms. The symbionts of legumes include the Rhizobiaceae, of outmost importance for sustainable agriculture as they are capable of fixing in nature up to 600-700 kg, and 150-200 kg in cropped land, of dinitrogen per ha and year. This family of alpha-proteobacteria embraces actually 17 genera and 168 species and is under taxonomic revision. Rhizobia have complex pan-genomes. Some species also have large plasmids or symbiosis islands, which are crucial for fitness, nodulation and N_2 fixation (pSym). The production and use of Rhizobia is shared at international level in all continents. A new challenging aspect of Rhizobiaceae and other dinitrogen fixing bacteria is that they can stimulate the plant growth *via* their ability to produce phyto stimulatory compounds. Indeed, these bacteria are marketed also as microbial biostimulants within and outside the European Union. They can be marketed and used as part of microbial consortia which include actinobacteria, saprophytic microfungi and mycorrhizal fungi.

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Bacterial production of indole-3-acetic acid (IAA) and its effect on nitrogen fixation in different host plants

C. Bianco, M.L. Amenta, S. Varriale, S. Milano, and R. Defez

Institute of Biosciences and Bioresources (IBBR), Naples, Italy

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Research on biological nitrogen fixation (BNF) by diazotrophic bacteria able to reduce the atmospheric N to ammonium using nitrogenase enzyme systems, has greatly expanded by the discovery of N-fixing bacterial endophytes inhabiting the internal plant tissues of non-nodulating plants. Indole-3-acetic acid (IAA) is the major plant auxin and plays a key role in plant growth and development. IAA production and secretion is widely distributed in bacteria inhabiting diverse environments, including soil and water, and in plant and animal hosts.

IAA production by microbes establishing beneficial interactions with plants can positively influence various metabolic and physiological processes, which lead to improved growth of the

host plant. These benefits include increased nutrients uptake, reduce susceptibility to abiotic and biotic stresses, and enhanced nitrogen fixation. We demonstrated that the overexpression of IAA in rhizobia triggered nitrogen-fixation, ameliorated adaptation to stressful environment, and stimulated of C and N metabolism, both in free-living bacteria and host plants. We have also shown that the positive effect of bacterial IAA overproduction on nitrogen fixation was not restricted to rhizobia-legumes symbiosis, but it was a more general phenomenon which included cereal-endophyte associations. Since transgenic plants overexpressing IAA or other hormones might have an imbalance affecting their development and differentiation, we suggest that the use of transient system, consisting of rhizobia or endophytic bacteria able to produce high levels of IAA in root nodules or other plant tissues, could be a successful strategy to improve the nitrogen fixation efficiency and stress response of the host plants.

Genetics of nitrate transport and signaling in N-fixing symbioses

V.T. Valkov[1], A. Barbulova[1], A. Notte[1], C. Codano[1], A. Rogato[1], S. Radutoiu[2], B. Lacombe[3], **M. Chiurazzi[1]**

[1]Institute of Biosciences and Bioresources, CNR, Naples, Italy

[2]Department of Molecular Biology and Genetics, Aahrus University, Aahrus, Denmark

[3]IPSIM, Univ. Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

The biological N_2-fixation which evolved in legume plants represents an objective advantage owing to the capacity of converting atmospheric N_2 into plant-assimilable NH_3. However, both formation and functioning of N_2-fixing nodules require high amounts of carbon and energy, and therefore, it is not surprising that legumes have developed finely tuned mechanisms to regulate nodule formation, development and functioning in relation to the N demand of the plants. In particular, when a N source is available in the rhizosphere, nodule formation capacity declines as well as the efficiency of existing N_2-fixing nodules. So far, the investigation of the role of nitrate as regulator of the symbiotic N_2-fixation has been mainly limited to the inhibitory effects exerted by high external concentrations on nodule formation, development and functioning. Nitrate transporters are obvious candidates for playing important roles in the control of nitrate signaling during the legumes-rhizobia symbiotic interactions. The main nitrate transporter protein families in plants are represented by the low-affinity Nitrate Transporter Peptide (NPF) and the high-affinity Nitrate Transporter (NRT2). NPF is a large family of 53, 80 and 86 members in *Arabidopsis thaliana*, *Oryza sativa* and *Lotus japonicus*, respectively. We have recently functionally characterized three *L. japonicus* nitrate transporters, LjNPF8.6, LjNPF3.1 and LjNRT2.4 playing positive roles in the control of nitrate flux through cytosolic and symbiosome compartments of mature *Lotus* nodules. This route of nitrate appears to be particularly important under hypoxic conditions when a nitrate-dependent respiratory chain must be achieved to support the energy status for an efficient nodule functioning.

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Plenary Session 2
Metabolic adaptation to stress conditions

The holobiont approach in health and disease

F. Rohwer

San Diego State University, CA, USA

Viruses, and particularly phage that infect bacteria, are the most abundance and diversity life forms on the planet. Given their success throughout the biosphere, it is expected that phage are essential members of the animal and plant holobionts. We have shown that phage form a bacterial selective, adaptive immune system that helps protect the mucosal surfaces of animals and establish the microbiome. Additionally, phages are actively transported across epithelial layers and may provide a systemic protection against bacteria. These two findings strongly suggest that phage formed the first acquired immune system and they remain important in extant animal immunology. We are currently leveraging our understand of these complicated interactions between phage, microbes, and human hosts to improve phage therapy.

Metabolic changes of *Neisseria meningitidis* during host infection

P. Alifano

Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy

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Metabolic adaptation to host microenvironments and underlying regulatory mechanisms play a critical role in invasive meningococcal disease. *Neisseria meningitidis*, a narrow host range *Neisseria* species, is able to use very few compounds including lactate, pyruvate, glutamate, glucose and maltose, which are present at very different ratios in microenvironments relevant to meningococcal infection. Glucose is the predominant carbon source in blood; by contrast, lactate is the major carbon source during growth in the cerebrospinal fluid, as well as in the nasopharynx. Lactate and pyruvate tend to be used as major carbon sources within phagocytic cells. Glutamate may represent an important carbon source not only in the intracellular environment, but also in cerebrospinal fluid where the levels of this amino acid strongly increase during the meningitis and correlate with disease severity. The uptake of glutamate is considered critical for meningococcal infection in both cell and animal infection models and is also instrumental in preventing oxidative injury, as glutamate is the precursor of glutathione. Moreover, a large genomic island allows *Neisseria meningitidis* to utilize propionic acid by methylcitrate cycle also attenuating its toxicity, with implications for colonization of the human nasopharynx. Indeed, *N. meningitidis* colonization seems to be correlated with colonization of propionic acid generating bacteria. Here we discuss on how the metabolic adaptation of *N. meningitidis* has evolved relative to other human-dwelling *Neisseria* species, and we focus on the methylcitrate cycle as specific to pathogenic *Neisseria* species and whose main role may be to challenge oxidative stress in specific host microenvironments.

Polyamine metabolism during Shigella infection

G. Prosseda
Sapienza Università di Roma, Italia

Polyamines are a class of molecules comprising linear or branched aliphatic chains characterized by at least two terminal amine groups. The simplest and most common natural polyamines are putrescine, spermidine, spermine, and cadaverine.These molecules are essential for the growth and viability of eukaryotic and prokaryotic cells, and due to their involvement in several important biological processes, their cellular levels are tightly controlled by metabolic regulation and transport. Shigella, the etiologic agent of human bacillary dysentery, reaches, invades, and multiplies within human colonocytes through a multistep and complex mechanism, including survival within macrophages and induction of their pyroptosis. Shigella exhibits some differences compared to that of its ancestor, E. coli. In essence, Shigella does not synthesize cadaverine and accumulates spermidine as a result of the inactivation of the cadA gene, which encodes lysine decarboxylase, and the speG gene, which encodes acetyl spermidine transferase. The loss of these functions contributes to optimizing Shigella virulence, and for this reason, mutations affecting the cadA and speG genes are called patho-adaptive mutations. Spermidine accumulation increases Shigella resistance to oxidative stress by enhancing the expression of catalase encoded by the katG gene. Nevertheless, we report results showing a more complex scenario in which polyamines may play an intriguing role in bacterial-host interactions, particularly in Shigella's ability to modulate the macrophage response to its invasion.

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Fighting *Staphylococcus aureus* through iron starvation

E. Frangipani
Department of Biomolecular Sciences, University of Urbino Carlo Bo, (PU), Italy

Antimicrobial resistance (AMR) is one of the top 10 global public health threats, responsible for more than 700,000 annual deaths, a scenario expected to rapidly worsen, especially due to antibiotic overuse/misuse during the Covid-19 pandemic.
Staphylococcus aureus (Sa) is an opportunistic bacterial pathogen frequently involved in multi-drug-resistant, healthcare-associated infections, warranting the development of novel therapeutic options.
Almost all living organisms have an absolute need of iron to fulfill a plethora of biological functions. In the human host iron is sequestered by iron- and heme-binding proteins, thus limiting its availability to invading bacteria, as part of a defense mechanism called Nutritional Immunity. However, successful pathogens, including *Sa*, have evolved various mechanisms to counteract the host iron-withholding capacity, including the secretion of proteases and the production of siderophores and hemophores.
This talk will illustrate the main strategies possessed by *Sa* to acquire iron, and their role during host invasion and pathogenesis, as well as the approaches that can be put in place to inhibit them. My group is the recipient of a grant (PRIN2020AE3LTA) within the ERASE multidisciplinary project, aiming at characterizing both hemophore- and siderophore-mediated iron acquisition by *Sa* and design small molecules to block these mechanisms. The generation of *Sa* mutants devoid of iron-uptake systems as well as the setting of culturing conditions to assay their inhibition and evaluate their contribution to bacterial growth, are currently ongoing in our laboratory. Targeting *Sa* iron acquisition at different sites will represent a novel promising antibacterial strategy to fight the current and worrisome AMR.

Plenary Session 3
Cell envelope remodeling to shape bacterial fitness in response to the host and environment

One or two membranes? Illuminating the evolution of the cell envelope across the Tree of Bacteria

S. Gribaldo

Department of Microbiology, Institut Pasteur, Paris, France

The envelope is one of the oldest and most fundamental cell components, and the first interface between an organism and its environment. While a great diversity of envelopes exists in the three domains of life, Bacteria are unique in having either one membrane (Gram-positives or monoderms) or two membranes (Gram-negatives or diderms). How the transition between these two radically different envelopes occurred has been one of the most intriguing mysteries in evolutionary biology. We have recently tackled this question by merging large-scale phylogenomics approaches with the development of a new experimental diderm model belonging to the classical monoderm Firmicutes. Our results shake longstanding assumptions and show the power of continuous exploration of ever larger fractions of microbial diversity.

Peptidoglycan remodelling and bacterial fitness under stressful conditions

V.M. Hernández-Rocamora[1], F. Corona[1^], T. Knowles[3], W. Vollmer[1,2*]

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The envelope of diderm bacteria comprises two lipid membranes surrounding a layer of peptidoglycan or sacculus. The sacculus maintains the shape of the cell shape and together with the outer membrane (OM) protects it from bursting by the turgor. The OM is asymmetric; its external leaflet contains lipopolysaccharide and the internal leaflet phospholipids. The OM protects the cell against many antimicrobials because the abundant β-barrel proteins do not allow the entry of these into the cell. The cell envelope is in constant change as the cell grows and divides, with the peptidoglycan being constantly remodelled for sacculus growth and assembly of envelope-spanning protein complexes such as flagella, secretion systems or lipid transport machineries. How peptidoglycan growth and remodelling is coordinated with outer membrane synthesis is coordinated is understudied currently. We aim to explore how key outer membrane synthesis processes such as LPS transport by the LPT system and β-barrel proteins assembly by the BAM complex are coordinated with peptidoglycan expansion. We have recently identified how the β-barrel protein assembly activity is confined to sites near new peptidoglycan biogenesis through the inhibition of BAM activity by old (mature peptidoglycan). We have now identified residues in the BAM complex component BamA interacting with peptidoglycan. Our results also shed light on whether transport of β-barrel proteins to the OM requires PG remodelling. Our work aims to gain an integrated understanding of cell envelope expansion in diderm bacteria under normal conditions and environmental or antibiotic stress conditions.

Peptidoglycan remodeling and amidases activation under lipopolysaccharide biogenesis defects

A.M. Martorana[1], M. Zaccaria[1], J. Biboy[2], L. Alessandrini[1], D. Hysa[1], W. Vollmer[2,3] and A. Polissi[1]

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The multi-layered envelope of Gram-negative bacteria is composed by the inner membrane (IM), the peptidoglycan cell wall and an asymmetric outer membrane (OM) containing Lipopolysaccharide in the outer leaflet. This complex structure behaves as a selective chemical barrier and allows cells to sustain large turgor pressures. In *Escherichia coli* additional robustness to the envelope is conferred by the covalent attachment of OM to PG mediated by Braun lipoprotein Lpp. Bacterial cells need to coordinate the growth of OM and PG layers to maintain cellular integrity during cell cycle and enable survival in challenging or stressful environments. In this context we are characterizing PG remodeling factors whose functions is absolutely required to avoid cell lysis when the OM asymmetry is lost because of disruption of LPS outer layer or defective LPS biogenesis. These factors include: i) the LD-transpeptidase LdtD and LdtE that introduce the non-canonical 3-3 cross-links in the PG layer, ii) the DpaA enzyme that detaches Lpp from the PG and iii) ActS a novel regulator of amidases, the enzymes that hydrolyze septal PG during cell separation. These factors appear to be part of a complex network where DpaA plays a central role in that not only directly controls covalent linkage between OM and PG but also indirectly modulates both ActS activity and 3-3 cross-link level in the cell. Our results support a model in which PG remodeling and OM biogenesis are coordinated to maintain cell viability under envelope stress conditions.

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Regulation of peptidoglycan synthesis during growth and division

O. Massidda

Università di Trento

Streptococcus pneumoniae (pneumococcus) is a clinically relevant Gram-positive pathogen of ovoid-shape morphology. To achieve and maintain its characteristic shape, the pneumococcus alternates sidewall and septal peptidoglycan (PG) synthesis during the cell cycle. Here, we will discuss the molecular mechanisms that link PG synthesis to the cell shape of *S. pneumonia*, through the identification and the characterization of the key players involved. Our findings provide a comprehensive model to understand the intricate relationship between these proteins and their role in the regulation of *S. pneumoniae* growth and division. This model not only deepens our understanding of the fundamental mechanisms governing the pneumococcal cell cycle but also provides valuable insights into potential targets for therapeutic interventions. By unraveling the molecular pathways involved in cell shape determination, we open up new avenues for the development of innovative strategies to combat *S. pneumoniae* infections.

Plenary Session 4
Functional microbial diversity in polluted environments

Microbial biosurfactants: Current trends and applications in environmental, agricultural, and biomedical industries

I.M. Banat

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Microbial biosurfactants are naturally occurring surface active molecules capable of partitioning at interphases reducing surface and interfacial tensions between different phases such as gas, liquid and solid. They can also act as foaming emulsification and wetting agents that have low critical micelle concentrations and surface-tension properties compared to their chemical counterparts. These compounds can potentially replace chemical surfactants which typically are more recalcitrant and problematic compounds to the environment. Glycolipidic and lipopeptides biosurfactants are the most promising types including sophorolipids produced by yeasts strains such as *Starmerella* and *Candida*, rhamnolipids produced by *Pseudomonas aeruginosa* bacteria and other related species, mannosylerythritol lipids produced by *Pseudozyma* yeasts and surfactin produced produced by *Bacilli* bacteria. Global interest in biosurfactants has been steadily increasing during the past three decades mainly stimulated by their favorable surfactant characteristics and environmentally friendly features including sustainable production technologies, biodegradability, and lower toxicity. Their main potential applications include use as environmental oil cleanup, combating agricultural phytopathogens as biopesticide particles, dispersant/solubilizing molecules for hydrophobic pollutants, use as components in both domestic and industrial cleaning products, anti-microbial adjuvants and/or agents, and as emulsifiers or probiotics compounds in food products. In addition, several medical and pharmaceutical applications including cosmeceuticals as anti-aging, oral hygiene products, wound-healing agents and in dermatological products, have been described. This presentation will discuss biosurfactants current trends, potential future applications, and challenges in the areas of environmental, agricultural, and biomedical related industries.

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Microbial ecosystem functions in polluted environments

I. Gandolfi

Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy

Air pollution in urban areas is a global concern because it is increasing worldwide due to rapid economic growth and has detrimental effects both on human health and ecosystem functioning. Plants are known to effectively contribute to the enhancement of ecosystem services in urban areas, including air pollution reduction, mainly through adsorption processes. In addition, microorganisms hosted by the phyllosphere, comprising the aerial parts of plants and dominated by leaves, have been suggested to effectively contribute to reduce air pollution levels in urban areas through biodegradation or transformation of pollutants adsorbed onto leaves. However, the actual extent of the potential for air remediation by phyllospheric bacteria is still largely unexplored. In fact, phyllospheric bacterial community structures often show both temporal and spatial dynamics and can vary among plant host species, due to complex interactions with plant physiology. A wider knowledge of phyllospheric bacterial communities hosted by urban trees and a better understanding of processes driving their assembly will help elucidating microbial roles in air pollution mitigation and quantifying the contribution of different plant-bacteria systems to air pollution-related ecosystem services.

Mycoremediation in TPH polluted environments: fungal and bacterial interactions

S. Crognale[1], D. Lelli[1], A. Firrincieli[1], F. Bagnato[2], F. Villani[2], R. Cicciarelli[2], M. Petruccioli[1], A. D'Annibale[1]

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The interactions between fungal and bacterial communities in the soil framework might include: physical interactions via dispersion of degradative bacteria by fungal hyphae, and chemical interactions, via degradation of organic contaminants, their co-degradation, and enhancement of degradative enzyme production and secondary metabolites.

In bioremediation, which involves co-metabolism and a hierarchical relationship of contaminant degradation among different microbial populations, there are many expectations on fungi and bacteria to remove toxic recalcitrant compounds in a sustainable and synergistic manner. In the field of mycoremediation, where fungal organisms are inoculated to degrade toxicants in soils, several examples of positive interaction between bacteria and fungi have been reported and a brief overview of case studies conducted on historically contaminated soils will be presented in this respect.

Mycoremediation is a very widely studied technology at lab-scale and there exists a few experiences at bench-scale, a paucity of information is currently available regarding its scale transfer for further commercialization. In this context, the LIFE MySOIL project (https://lifemysoil.eu) aims to demonstrate the feasibility of mycoremediation for total petroleum hydrocarbons (TPH) removal technology at pilot and full scale, moreover the project offers an ideal platform to study and elucidate interaction mechanisms between indigenous microbiota and fungal inocula.

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An experimental set-up, based on 6 different bioaugmentation mesocosms inoculated with *Pleurotus ostreatus* or *Pleuroutus* spent compost, has been used to monitor the fungal and bacterial dynamics along with TPH degradation process through metagenomics-based approaches, and investigate the metabolic potential of the indigenous community involved in TPH degradation.

Benthic microbial response to pollutants in anthropized marine sediments

E. Tamburini

Department of Biomedical Sciences, University of Cagliari, Italy

The Mediterranean Sea is an interesting case study for investigating the impacts of anthropogenic pressures on marine ecosystems as it combines numerous activities and strong demographic pressures. Moreover, the responsiveness of marine ecosystems to human pressures is accelerated by the oceanographic conditions of a semi-enclosed sea. The basin hosts more than 600 commercial ports and terminals and almost half of them is located in Greece and Italy. Being hot spots of contamination by multiple sources, ports represent one of the most challenging environments for understanding the links between pollutants and biological diversity. Currently, the analyses of sediments and associated benthic biota are the most used approach to develop ecological quality indexes and benthic microbial communities are gaining increasing attention in ecological monitoring. Based on these premises, the talk will present results obtained in multidisciplinary studies aimed at systematically investigating sediments collected from ports located along the Mediterranean coasts, with the final objective to develop tools for port sustainable management. The structure and composition of microbial communities were

assessed by targeted metagenomic analysis of the rRNA genes, and the links of communities with environmental and pollution variables were statistically investigated. Bioremediation tests under controlled conditions were performed comparing the performances of different remediation technologies on sediments from different locations. Finally, other examples of benthic microbial communities in coastal sediments impacted by anthropic pressures will be discussed. Overall, current knowledge is providing the foundation for integrating benthic microbiota in environmental monitoring, strategies for source pollution control, and planning of remediation intervention.

Plenary Session 5
Novel therapeutic approaches against viruses

CRISPR-Cas gene editing approaches to attack the HIV provirus in the cellular reservoir

B. Berkhout
University Medical Centers, Amsterdam, NL

No abstract available

New therapies on the horizon for hepatitis B: are we close to a cure?

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Department of Pharmacological and Biomolecular Sciences (DiSFeB), University of Milan, Italy

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Hepatitis B virus (HBV) is a major cause of liver disease, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The current standard of care for chronic HBV infection is pegylated interferon-alpha (pegIFNα) or a nucleos(t)ide analog (NA). These therapies can suppress HBV replication and improve the patient's quality of life, but they do not completely cure the infection. This is because the virus can hide in the nucleus of infected cells in the form of stable covalently closed circular DNA (cccDNA). While a "sterilizing cure" that eliminates the viral genome from the body is difficult or even impossible to achieve in most patients, there have been major efforts to develop a "functional cure" for HBV. This would involve the sustained suppression of HBV replication and viral protein production, and possibly the restoration of the immune response to HBV. To this end, a number of novel direct-acting antivirals (DAAs), such as those targeting virus entry or capsid assembly, are in clinical trials. In addition, viral gene silencing by RNA interference or antisense RNA oligonucleotides, as well as immune modulatory strategies to stimulate adaptive or innate immunity and/or to remove immune blockade, are being tested.
In this lecture, I will discuss the current state of the art in the search for a cure for hepatitis B. I will review the different approaches that are being explored, and I will discuss the challenges and potential benefits of each approach. I will also discuss the latest research findings in this area, and I will provide an overview of the future of HBV cure research.

Exploiting Ebola virus suppression of the innate immune activation as target for drug development

A. Corona

Laboratorio di Virologia molecolare, Dipartimento di Scienze della Vita e dell'Ambiente. Università degli Studi di Cagliari. Cittadella Universitaria di Monserrato, Italia

The Ebola virus (EBOV) is a highly infectious and lethal pathogen responsible for recurrent clusters of Ebola virus disease (EVD), whose outbreaks have become increasingly frequent and large. Even with several promising therapeutic candidates under investigation, there is a lack of agents to treat the infection effectively. Therefore, efforts are devoted to elucidating all the possible targets and mechanisms to find new therapeutic opportunities. The EVD onset is linked to the ability of EBOV to efficiently suppress the innate immune system at the early stages of infection.

The main factors in this suppression are viral protein 35 (VP35) and viral protein 24 (VP24). VP35 is a multifunctional protein which plays a major role in the EBOV life cycle as a potent immune antagonist by inhibiting the dsRNA-induced activation of the RIG-I signalling pathway, suppressing IFN-beta production, while VP24 exerts its action by binding the NPI-1 subfamily of karyopherin-α proteins, involved in the nuclear transport of phosphorylated STAT1 protein, thus blocking the transcription of IFN-stimulated genes.

In this lecture I will present how molecular virology and cell-based mechanistic studies revealed how both VP35 and VP24 proteins can be efficiently targeted, identifying ligands able to restore the innate immune response, and how the restoration or pharmacological amplification of the innate immune response has been shown to overcome the EBOV replication.

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Phage genomics and therapy

R. Lavigne

University of Leuven, Belgium

No abstract available

PLenary Session 6
Bacterial physiology: back to the future of Microbiology

Redox condition adaptation, energy metabolism and antibiotic sensitivity in *Escherichia coli*

F. Barras

Institut Pasteur, Paris, France

In the gut, *Escherichia coli* multiplies in different redox environments. Accordingly, it relies on different mode of growth, from anaerobic fermentation to anaerobic or aerobic respiration. In a first part, I will present how these different conditions influence the level of phenotypic resistance of *E. coli* to aminoglycosides and discuss the positive role that a AAA+-ATPase, RavA ViaA, plays under anaerobic fumarate respiratory conditions. Respiratory chains rely on electron transfer via quinone carriers. In a second part, I will present a recently dogma-breaking finding on ubiquinone biosynthesis under anaerobiosis. Last, besides acting as an electron acceptor, fumarate is a so-called oncometabolite and can modify cysteine residues. This is the basis of multiple pathologies in human. In a third part, I will present new results aiming at assessing the effect of fumarate analogs, routinely use in medicine, on *E. coli* proteome and on microbiota dynamic.

Bacterial iron metabolism; turning basic physiology into drug development

P. Visca

Department of Science, Roma Tre University, Italy

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Iron is essential for almost all forms of life, but it is poorly available due to low solubility and entrapment by iron-binding (macro)molecules. Therefore, iron must be transported across the membrane(s) through specific active transport systems. Bacteria, fungi, and some plants have evolved the ability to produce, release, and uptake high-affinity iron-binding small molecules called siderophores, belonging to different chemical classes. Despite species-specific chemical diversity, siderophore biogenesis, transport, and regulation are governed by similar rules in prokaryotes; they are produced and taken up only when the cytoplasmic iron concentration is too low to sustain the cellular metabolism. Repression under conditions of iron sufficiency is a dogma of iron uptake regulation since too much iron would be lethal due to oxidative stress. However, positive control of iron uptake is also observed, especially in species that can prey upon different exogenous iron carriers, so exogenous carriers are sensed, and cognate transporters are expressed only when a given carrier is available.

In bacterial pathogens, iron scarcity is also an environmental signal of their transition into the host, where iron-binding proteins contribute to innate immunity by depriving the invading pathogen of an essential metal. It is now clear that sensing low iron levels *in vivo* serves as an inducive stimulus for the expression of some virulence factors by bacterial pathogens, and that both nutrition and virulence are closely linked to bacterial iron uptake capabilities and metabolism. Therefore, both iron uptake systems and iron metabolism have become attractive targets for the development of novel antimicrobials against multidrug-resistant critical pathogens. Recent advances in the discovery of novel antimicrobials like iron chelators, iron mimetics, and siderophore-antibiotic conjugates provide a paradigmatic example of how basic knowledge of bacterial physiology can be turned into promising antibacterial strategies.

At the crossroad of metabolism and bacterial pathogenesis: the case of PE and PPE proteins of *M. tuberculosis*

G. Delogu
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No abstract available

Analysis of host-pathogen interaction by dual RNA sequencing: methods and applications

F. Santoro
Università di Siena, Italia

No abstract available

PHD DAY PARALLEL SESSIONS: short talks from selected abstracts

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Session A - Microbial genetics and genomics

Chairs: **M. Ventura, E. Rossi**

A1 Prophage Φ1207.3 is responsible for a temporary activation of a mutator phenotype in *Streptococcus pneumoniae* upon irradiation at different UV-C light wavelengths

C. Apicella, C. Petri, M. Tirziu, L. Colombini, E. Lazzeri, A.M. Cuppone, F. Santoro, G. Pozzi, F. Iannelli

Laboratory of Molecular Microbiology and Biotechnology (LAMMB), Department of Medical Biotechnologies, University of Siena, Italy

The SOS response is an inducible system activated by some bacterial species in the presence of stress and extensive DNA damage. Streptococci lack a canonical SOS response, but an SOS-like response was reported in some species. The *mef*(A)-*msr*(D)-carrying prophage Φ1207.3 of *Streptococcus pyogenes* contains a region, spanning *orf6* to *orf11*, showing homology to characterized streptococcal SOS-like cassettes. We constructed isogenic *Streptococcus penumoniae* strains to investigate whether this cassette confers an SOS-like response activation after UV-C light exposure. Upon exposure to UV fluences ranging from 0 to 6400 J/m2 at four different wavelengths, 255, 265, 275 and 285 nm, we found that the presence of Φ1207.3 SOS-like cassette increases bacterial survival up to 34-fold. Mutation rate was determined by measuring rifampicin resistance acquisition upon exposure to UV fluence of 50 J/m2 at the four wavelengths by fluctuation test. The presence of Φ1207.3 SOS-like cassette resulted in a significant increase in the mutation rate (up to 18-fold) at every wavelength. In conclusion, we demonstrated that Φ1207.3 carries a functional SOS-like cassette responsible for an increased survival and increased mutation rate in *S. pneumoniae*.

A2 Functional characterization of the RS04555 gene and its association with persistent cystic fibrosis lung infections of *Pseudomonas aeruginosa*

V. Baldelli[1], S.J. Carrasco[1], H.K. Johansen[2,3], S. Molin[2], M. Paroni[1], P. Landini[1], E. Rossi[1]

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Pseudomonas aeruginosa is the leading cause of death in cystic fibrosis (CF) patients. Due to its ability to withstand antibiotic treatments, lung infections often become difficult to treat long-term chronic infections. Hence, identifying novel mechanisms underlying *P. aeruginosa* persistence can be effective for treatment development.

Although gene expression analysis in CF airways revealed a common transcriptional program shared by *P. aeruginosa* populations, several genes with an altered expression are poorly characterized and might represent novel persistence determinants and targets for antimicrobial strategies.

Notably, the gene RS04555 is conserved in human-infecting *P. aeruginosa* strains and is homologous to the virulence-related *sirB* gene of *Salmonella enterica* serovar Typhimurium. *In silico* promoter analysis, coupled with genetics and biochemical approaches, revealed that the global virulence regulator Vfr, and the alginate and motility regulator AmrZ are repressors of RS04555, suggesting its importance also in *P. aeruginosa* virulence. By testing RS04555 knockout mutant on important virulence phenotypes for *P. aeruginosa*, we identify this gene as a modulator of both virulence and biofilm. Further, qRT-PCR showed a differential expression of the *rsmY/W/Z* sRNA, indicating that the virulence modulation might occur by altering the Gac/Rsm pathway. Finally, the lack of RS04555 leads to the emergence of *P. aeruginosa* small colony variants, often associated with *in vivo* persistence and deriving from the accumulation of genetic mutations as suggested by our whole-genome sequencing analysis.

Overall, our results suggest that mining the *in vivo* gene expression data allows the identification of previously unknown determinants essential for *P. aeruginosa* pathogenicity.

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A3 Antibiofilm activity of a *Staphylococcus aureus* phage: potential key role of its baseplate protein

C. Campobasso[1,2], J. Wagemans[2], A. Tavanti[1], R. Lavigne[2], M. Di Luca[1]

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The efficiency of phages in targeting and killing bacteria could be impaired when cells are biofilm-embedded. Our work aimed to drive the evolution of phage Romulus (*Silviavirus* genus) within *S. aureus* biofilm and to analyse the mutations occurred in the evolved phage clones.

A one-month directed evolution protocol was based on a one-hour phage incubation with a pre-formed biofilm on porous beads, followed by an eight-hour bead incubation in fresh BHI broth. At the end of each cycle, the medium containing phages was collected and used in the following round. After the 31st round (R31), ten plaques (p1 to p10) were isolated and sequenced. The antibacterial effect of the wild type (wt), the R31 and the single mutant phages was assessed against planktonic (multiplicity-of-infection MOI 0.1) and sessile cells (from 10^7 to 10^9 PFU/ml), after 24 hours.

No reduction of planktonic cells was observed with the wt phage compared to the untreated control. Conversely, the eradication was obtained with R31 cocktail, p2 and p5 phages. In comparison to the wt phage activity, a statistically significant biofilm reduction (at least 3log10) was achieved with evolved phages, when tested at 10^8-10^9 PFU/ml. Genome sequencing revealed that most of the mutations occurred in gp58, annotated as baseplate protein, which is predicted to have depolymerase activity (98.0% certainty, by PhageDPO).
These results suggest a key role of the baseplate protein (as putative depolymerase) in targeting bacterial cells, including those embedded in biofilm. Ongoing experiments are aimed at better understanding gp58 function.

A4 A new drug candidate against *Mycobacterium abscessus* and other cystic fibrosis pathogens

G. Degiacomi[1], L.R. Chiarelli[1], L. Muñoz[2], O. Riabova[3], N.I. Loré[4], G. Stelitano[1], V.C. Scoffone[1], D. Recchia[1], N. Monakhova[3], F. Saliu[4], U. Postiglione[1], J.M. Ezquerra Aznárez[2], A. Griego[5,6], G. Ciniero[1], E. Scarpa[5,6], L. Rizzello[5,6], S. Buroni[1], D. Sassera[1,7], D. Cirillo[4], S. Ramon-Garcia[2,8], E. Tortoli[4], V. Makarov[3], M.R. Pasca[1,7]

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VOMG is a new molecule with high bactericidal activity against *Mycobacterium abscessus* (Mab) (MIC=0.25 µg/ml) which is the pathogen of greatest concern among non-tuberculous mycobacteria (NTM) due to its intrinsic drug resistance and poses a threat especially to individuals with cystic fibrosis (CF).
VOMG is also active against Mab biofilm and other NTMs including multi-drug resistant clinical isolates and other CF pathogens, such as *Staphylococcus aureus* and *Acinetobacter baumannii*. *In vitro* studies have proven that VOMG can be used for combination therapy. A SAR has been established with drug-like ADME and, due to its physicochemical properties, it is suitable for new drug delivery, including aerosol inhalation. VOMG has an activity in Mab-infected mice similar to that of amikacin, the comparator drug, and is non-toxic by intragastric administration in healthy mice, with high bioavailability.
Interestingly, transcriptomic analysis revealed that VOMG treatment inhibits essential metabolic pathways, such as cell division. It was shown to inhibit Mab and *S. aureus* FtsZ activity by blocking its polymerization: single-cell analysis confirmed this mechanism of action.

Further preclinical studies are ongoing for VOMG progression.
VOMG was patented under the co-ownership of UNIPV and Fondazione Italiana Ricerca Fibrosi Cistica (FFC). This work was supported by FFC (18/2021, 14/2020, 19/2018) and EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

A5 Structural and functional analysis of the active cow rumen's microbial community provides a catalogue of genes and microbes participating in the deconstruction of cardoon biomass

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Background. Ruminal microbial communities are a renowned source of microorganisms and enzymes highly specialized in deconstructing lignocellulose, a principal component of plant biomass and currently considered an important bottleneck in the development of second-generation biofuels and bioproducts. Cardoon (*Cynara cardunculus*) is a promising inedible energy crop for current uses in biorefineries and the emerging bioenergy and bioproducts industries. In this study, we used the nylon bags technique to dissect the ruminal metatranscriptome and identify bacteria and fungi, and their fibrolytic enzymes, highly active in the deconstruction of cardoon lignocellulose.

Results. While the rumen and cardoon-enriched microbial communities were similar in terms of composition, significant differences between the two communities were observed with respect to the expression of transcripts involved in the fermentation of sugars and methanogenesis. Likewise, transcripts encoding for fibrolytic enzymes, which accounted for 1.5% (7,394) of the total RNA coding fraction, were mostly affiliated to renowned rumen cellulolytic microorganisms. However, the comparison of their expression profile between the rumen fiber-adherent microbial community and the community enriched in nylon bags, highlighted that *Oscillospiraceae* and *Neocallimastigaceae* fibrolytic enzymes were responsible for the early deconstruction of cardoon plant cell wall polysaccharides, while *Lachnospiraceae* and *Treponemataceae* fibrolytic activity persisted up to 96 h incubation, were only the most recalcitrant fraction of the cardoon fibres was left in the nylon bags.

Conclusions. Metatranscriptomic sequencing data revealed that the cow rumen microbiome harbors a repertoire of enzymes and microorganisms potentially exploitable for the production of biofuels and high-value bioproducts from cardoon.

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A6 The infant gut microbiota atlas: detailed insights from composition to functional microbe-based biodiversity

G.A. Lugli[1], L. Mancabelli[2,3], C. Milani[1,3], F. Fontana[1], C. Tarracchini[1], G. Alessandri[1], D. van Sinderen[4], F. Turroni[1,3] and M. Ventura[1,3]

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During infancy, gut microbiota development is a crucial process involved in establishing microbe-host interactions that may persist throughout adulthood and are believed to influence host health. To fully understand the complexities of such interactions, it is essential to assess the gut microbiota diversity of newborns and its associated microbial dynamics and relationships in health and disease. In this context, 10,935 shotgun metagenomic datasets were taxonomically and functionally classified to explore microbial biodiversity during the first three years of human life. Microbial species distribution between infants revealed the presence of eight major Infant Community State Types (ICSTs), dominated by 17 bacterial taxa, whose distribution corresponded to the geographical origin and infant health status. In total, 2390 chromosomal sequences of the predominant taxa were reconstructed from metagenomic data and used in combination with 44,987 publicly available genomes to trace the distribution of microbial Population Subspecies (PS) within the different infant groups, revealing patterns of multistrain coexistence among ICSTs. Finally, the implementation of metagenomic- and metatranscriptomic-based metabolic profiling highlighted different enzymatic expression patterns of the gut microbiota that allowed us to acquire insights into mechanistic aspects of health-gut microbiota interplay in newborns. Comparison between metagenomic and metatranscriptomic data highlights how a complex environment like the human gut must be investigated by employing both sequencing methodologies and possibly supplemented with metabolomics approaches. While metagenomic analyses are very useful for microbial classification aimed at unveiling key players driving microbiota balances, using these data to explain functionalities of the microbiota is not always warranted.

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A7 RsaL-driven negative regulation promotes heterogeneity in *Pseudomonas aeruginosa* quorum sensing

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Quorum sensing (QS) is canonically regarded as an intercellular communication system allowing bacterial cells to synchronize gene expression and hence to collectively perform tasks in response to cell density. QS has long been considered as a process synchronously and homogeneously activated by the entire population, as it usually regulates bacterial collective behaviors. However,

growing evidences revealed that cell-to-cell variation in QS activation can occur, often resulting in coexisting subpopulations of quorate (QS ON) and non-quorate (QS OFF) cells sharing the same environment. Such phenotypic heterogeneity has been recently observed also in the activation state of las QS system in the opportunistic pathogen *Pseudomonas aeruginosa*. However, the molecular mechanism(s) underlying this phenomenon have not yet been defined.

Here, to provide a mechanistic explanation to *P. aeruginosa* QS heterogeneity, single-cell level analyses *via* confocal microscopy imaging were performed on *ad hoc* designed biosensor strains, in which *las* system activation results in fluorescence emission. Results showed that activation of the *las* QS system does not occur synchronously in all cells of the population, with different fractions of quorate and non-quorate subpopulations co-existing even at high cell density. In addition, we demonstrated that the heterogeneous activation of the *las* system arise at the transcriptional level, through the action of the RsaL negative regulator. In accordance, we observed an inverse correlation between *rsaL* expression levels and las QS activation in single cells. Interestingly, the *rhl* QS system, that is not controlled by an analogous RsaL protein, showed higher homogeneity with respect to the *las* system.

A8 Improved antimicrobial activity of colistin in combination with putative ArnT inhibitors in *Pseudomonas aeruginosa* biofilms

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Biofilm is an adaptive strategy by which bacterial cells become embedded within an extracellular matrix that mediates surface adhesion and decreases drug penetration. In this environment tolerance to many antibiotics, including colistin, is favored. Colistin, which is the last-resort therapeutic option in infections by multi-drug resistant pathogens Gram-negative bacteria, interacts electrostatically with their outer membrane (OM) leading to membrane destabilization, increased permeability, and cell death. Colistin resistance is mediated by OM remodeling, which in *Pseudomonas aeruginosa* is mainly mediated lipid A aminoarabinosylation, whose last step is catalyzed by the aminoarabinosyl transferase ArnT. We have previously demonstrated that putative inhibitors of ArnT potentiate the activity of colistin against colistin resistant *P. aeruginosa* strains (doi:10.1093/jac/dkaa200; doi:10.1021/acs.joc.0c01459). Taking into consideration that the activity of colistin is impaired in biofilms, we have analyzed the expression of ArnT in biofilms, as compared to planktonic cells, and the colistin adjuvant activity of two putative inhibitors of ArnT, FDO and its derivative FDO-H. Biofilms were developed from two different colistin resistant strains, one *in vitro* evolved reference strain and one clinical isolate. Interestingly, ArnT expression appeared higher in biofilms respect to planktonic cells. Additionally, biofilms treated with colistin in combination with FDO or FDO-H showed reduced metabolic activity and viable cells counting respect to samples treated with colistin-only. Collectively, our data suggest that upregulation of ArnT may contribute to the reduced colistin activity in biofilms and that FDO and FDO-H potentiate colistin activity against *P. aeruginosa* biofilms.

A9 Charcaterization of carbohydrate active genes carried by an intestinal isolate of *Bacillus subtilis*

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SF106 is an aerobic spore former strain, previously isolated from ileal biopsies of healthy human volunteers[1], and shown to belong to *B. subtilis* species. SF106 has been proposed as probiotic strain for its ability to produce antimicrobials and vitamins and efficiently bind mucin and human epithelial cells[2]. A comparative genomic analysis of SF106 and PY79, the reference strain of the same species, indicated the presence only in SF106 of a 16,5 kb chromosomal region, characterized by a GC content similar to the rest of the chromosome and apparently not presenting insertion sequences at its boundaries. Such region contains a five-gene operon presumably involved in the utilization of carbohydrates with genes coding for a putative glycosyltransferase (GT2) and a putative glycoside hydrolase (GH126). A knock-out mutant lacking the entire operon was constructed and analyzed. Mutant spores showed an altered surface morphology, apparently lacking of the outermost exopolysaccharide layer. Those spores were less efficient than the isogenic wild type (wt) strain in binding human mucin. In addition, the mutant was also less efficient that the wt in adsorbing heterogous proteins. Experiment to analyze the expression of the operon are in progress.

[1]*Fakhry et al. Characterization of spore forming Bacilli isolated from the human gastrointestinal tract.* J. Appl. Microbiol. *2008, 105, 2178–2186.*

[2]*Saggese et al. Comparative Genomics and Physiological Characterization of Two Aerobic Spore Formers Isolated from Human Ileal Samples.* Int. J. Mol. Sci. *2022, 23, 14946.*

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A10 AsmA-like proteins are essential but redundant for growth and cell envelope integrity in *Pseudomonas aeruginosa*

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BACKGROUND. The outer membrane (OM) is an essential structure of diderm bacteria that protects them from large and/or hydrophobic toxic molecules, including many antibiotics. The OM is composed of phospholipids and lipopolysaccharide in the inner and outer leaflets, respectively, and hosts integral proteins (OMPs) and lipoproteins. While the systems responsible for translocation and insertion of lipopolysaccharide, lipoproteins and OMPs have been elucidated, the mechanism(s) mediating transport of phospholipids to the OM has remained elusive for decades. Very recently, studies in the model organism *Escherichia coli* have proposed a role for AsmA-like proteins in this process.

OBJECTIVES. To broaden the characterization of AsmA-like proteins by verifying their relevance for OM homeostasis and phospholipid transport in the human pathogen *Pseudomonas aeruginosa*.

METHODS AND RESULTS. Homology search and Pfam analysis combined with structural predictions revealed that *P. aeruginosa* possesses seven AsmA-like proteins, six of which have properties compatible with phospholipid transport from the cytoplasmic membrane to the OM. Deletion of *asmA*-like genes in all possible combinations in the reference strain *P. aeruginosa*

PAO1, followed by growth assays in the absence or presence of OM perturbing agents or antibiotics, revealed that four AsmA-like proteins are redundantly essential for growth and OM integrity. This evidence was confirmed by generating rhamnose-dependent conditional mutants. Notably, while three of these AsmA-like proteins are also present and important for OM homeostasis in *E. coli*, the other is specific to *Pseudomonas*, thus expanding the range of AsmA-like proteins that might play essential role(s) in diderm bacteria.

A11 Critical role of CotE in spore coat proteins deposition at different temperatures of growth

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The key to understanding the biogenesis of complex cellular structures is to study the production and the interaction of the morphogenetic proteins which have the role of coordinating the assembly of macromolecular scaffolds. In this work, we examine the assembly of a multilayered proteinaceous shell, the coat, that helps to protect bacterial spores from environmental assault. *Bacillus subtilis* is the model system for the study of endospore-forming bacteria. Over 100 different proteins compose the coat of *B. subtilis* spore. Among the morphogenetic proteins, CotE plays a key role in the proper assembly of the coat by shaping a ring around the forming-spore and driving the correct assembly of coat components. Several studies demonstrate that the temperature of growth and sporulation affects spore physiological properties and induces a switch between two alternative types of spore surfaces. To explore further how the temperature of sporulation influences the functional organization of CotE and its interaction with other proteins in the construction of the proteinaceous multilayer, we analysed the physiological properties and surface structures of mutant *B. subtilis* spores produced at 25°C, 37°C and 42°C.

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A12 O antigen production sensitises *Escherichia coli* to bile salts – A plausible explanation for how *E. coli* K-12 lost its O antigen

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Escherichia coli K-12 is a model organism for bacteriology and has served as a workhorse for molecular biology and biochemistry for over a century since its first isolation in 1922. However, *E. coli* K-12 strains are phenotypically devoid of an O antigen (OAg) since early reports in the scientific literature. Recent studies reported the presence of independent mutations that abolish OAg biogenesis in K-12 strains that originated from the same source, suggesting unknown evolutionary forces have selected more than once for the loss of OAg during early strain propagation. Here, we show for the first time that restoration of OAg in *E. coli* K-12 strain MG1655 synergistically sensitises bacteria to vancomycin with bile salts (VBS). Suppressor mutants surviving lethal doses of VBS mostly contained disruptions in OAg biogenesis. We present data supporting a model where the transient presence and accumulation of lipid-carried OAg intermediates in the

bacterial periplasm interferes with peptidoglycan synthesis, causing growth defects that are synergistically enhanced by bile salts. Lastly, we demonstrate that continuous bile salt exposure of OAg-producing MG1655 in the laboratory, can recreate a scenario where OAg disruption is under strong selection. Hence our work provides a likely explanation for the long-held mystery of how *E. coli* K-12 lost its OAg and opens new avenues for exploring long-standing questions on the intricate network coordinating the synthesis of different components of the Gram-negative bacterial cell envelope.

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Session B - Environmental and industrial microbiology

B1	**The Southern Ocean microbial community structure: a response to climate change**

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The Southern Ocean is home to the world's strongest and deepest currents that control the circulation of seawater across all the oceans. It connects the three main ocean basins (Atlantic, Pacific and Indian) and creates a global circulation system that is largely driven by the Antarctic Circumpolar Current (ACC). This current flows from west to east around Antarctica and generates an overturning circulation by fostering deep-cold water upwelling and formation of new water masses, which exert an out-sized influence on Earth's heat balance and distribution of nutrients and carbon worldwide. These features make the Southern Ocean a key player in buffering the effects of climate changes. While the effect of climate changes on the physicochemical properties of the Southern Ocean have been extensively investigated and include dramatic transformations like water acidification, depletion of sea ice, enlarged stratification, only few studies report the impact of such changes on the Southern Ocean microbiome structure and ecosystem functioning. Here we will focus on the global importance of Southern Ocean microbiome giving more light on the response of microbial community, in terms of biodiversity, structure, dynamics and function, to the ongoing climate changes events. Moreover, we will present data obtained from surface water samples collected during the XXXII Antarctic Oceanographic campaign. Our results show a distinct succession of the dominant microbial phylotypes related to the environmental conditions and represent an important baseline for future studies on the response of epipelagic microbial communities to the climate changes.

B2 Long-distance dispersals govern the global biogeography of Vibrio spp. in the ocean

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Bacteria belonging to the genus Vibrio are found in marine and brackish waters around the world and are the dominant cause of infections and death in humans and animals from the marine environment. In this study, the global biogeography of Vibrio was investigated for the first time by analyzing 1500 marine metagenomic samples (ca. 50 terabases of data) collected in the global ocean across different depth (surface vs deep-water) and size-fractions (free-living vs plankton-attached) during the Tara Oceans expedition from 2009 to 2013. By employing a robust bioinformatic workflow we showed that Vibrio is the seventh most abundant bacterial genus in the ocean. Vibrio species including potential human pathogenic species were found to be globally distributed and showed highest abundance in the plankton-attached size fraction of surface waters of the ocean. Furthermore, by examining Vibrio metagenomic data in relation to global ocean surface circulation data derived from the Global Drifter Program (https://www.aoml.noaa.gov/phod/gdp/index.php), we provided evidence on the role of plankton and marine currents in the oceanic connectivity of Vibrio populations via large scale surface transport a global scale through specific routes or “biological corridors”. Overall, these data provide a new paradigm to explain global distribution patterns of Vibrio species and the worldwide spread of their associated diseases.

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B3 Towards a harmonized system for the monitoring of soil microbial biodiversity

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Healthy soils are able to provide as many ecosystem services as possible, and they are generally characterized by a diverse and resilient microbial community. To constantly monitor soil health and promote its sustainable management, in 2009 the European Land Use and Coverage Area Frame Survey (LUCAS) was established by the Joint Research Centre (JRC) of the European Commission (EC). Since 2018, it also evaluates soil biodiversity. However, comparability assessment of biodiversity data obtained from LUCAS and individual EU Member States

is still lacking. Indeed, many factors may lead to biases, from the sampling procedure to the computational analysis.

One of the aims of the European Joint Programme on Soil (EJP-SOIL) is to compare the EC with national biodiversity assessment methodologies to harmonize the analytical procedures.

Over the 2022 LUCAS sampling campaign, soils from 98 sampling points were collected across Italy. Of these, 17 points were also sampled following national strategies. For this preliminary study, DNA was extracted from the soils collected in these double sampling points and the bacterial V3-V4 16S rDNA and fungal ITS2 regions were sequenced.

Obtained data suggested that environmental variables have a strong significant effect on the soil microbial communities, while the different sampling strategy has a little or no effect. These data will be also compared with the EC results on the same LUCAS samples, to evaluate the effect of different analytical methods. This knowledge will help define standard procedures for setting up a national monitoring network and give clues for data comparison and harmonization.

B4 Study of a tellurite hyper-resistant mutant to delve into *Rhodococcus* stress response and resistance mechanisms to toxic metalloids

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Bacteria belonging to *Rhodococcus (R.)* genus can tolerate high concentrations of toxic metal(loid) s, such as tellurite (TeO_3^{2-}). The interaction mechanisms between metal(loid)s and bacteria have implications in several fields of environmental biotechnology such as i) remediation of polluted environments, ii) bioproduction of metal(loid)s nanostructures with biomedical (sensors) and industrial applications (quantum dots), and iii) recovery of rare metal(loid)s. Previous studies have documented the ability of *R. aetherivorans* BCP1 to reduce tellurite to its elemental form (Te^0), forming metal nanostructures that are less toxic than the oxyanion and recoverable from the environment. The molecular mechanisms underlying the high *Rhodococcus* resistance/ tolerance to TeO_3^{2-} are still mostly unknown. This work focuses on the study of the interaction between *Rhodococcus* cells and tellurite to delve into the mechanisms of stress response and metal biotransformation that allow high cellular resistance. With this purpose, a tellurite hyper-resistant mutant of *Rhodococcus aetherivorans* BCP1 (BCP1-Wh) was analysed. This mutant shows modified cell pigmentation and an improved tellurite resistance under both biofilm and planktonic growth conditions. We characterized BCP1-Wh to define the genetic determinant (Te^R gene) associated with this oxyanion resistance while detailed analyses of reactive oxygen species (ROS) production and tellurite uptake were performed to get insights into its stress response mechanisms. Finally, we have studied the phenotypes of the complemented BCP1-Wh and of a *R. opacus* PD630 mutant that was constructed by deleting an orthologous Te^R gene (identified in its genome) to confirm the association of this gene with tellurite resistance.

B5 Rhizoremediation potential in a historical polychlorinated biphenyl polluted site

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The Site of National Priority (SIN) Brescia-Caffaro is a highly polluted area in Northern Italy presenting historical mixed and uneven contamination by metals and organic pollutants, in particular polychlorinated biphenyls (PCBs) often exceeding the legal thresholds. Chemical and microbial profiling of the soils in the SIN agricultural areas demonstrated that pollutants were among the main drivers of soil microbiome assembly, allowing to speculate that bacterial degraders had been positively enriched during decades of natural attenuation. Due to the large site scale, rhizoremediation, relying on plant-mediated degraders' stimulation, was explored by testing at greenhouse level different combinations of plant species and soil treatments. Reed canary grass (Phalaris arundinacea L.) with/without soil periodic flooding, aimed to induce a redox cycle that would allow both reductive dichlorination and aerobic degradation, showed higher decrease of the original concentration of several PCB families in 18 months. Plant biostimulation of autochthonous microbial PCB degraders is a way to restore polluted sites where traditional remediation techniques are not sustainable, though its success requires the understanding of site-specific plant-microbe interactions. To this purpose, stable isotope probing (SIP) using 13C-labeled 4-chlorobiphenyl and 16S rRNA amplicon sequencing was applied on the biostimulated soil, to determine how the structure of total and PCB-degrading populations had been affected by the different treatments. Results showed different responses of bacterial taxa to specific rhizoremediation treatments and provided new insights into those active in PCB biodegradation, with Betaproteobacteria and Actinobacteria among the most abundant taxa deriving carbon from PCB.

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B6 Bioaugmentation-assisted phytostabilization of Sardinian abandoned mines by plant-growth promoter *Serratia* sp.

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Sardinian mining areas (SW Sardinia, Italy) represented one of the most important mining districts for Pb and Zn extraction at global level during the last two centuries. To address this issue, the utilization of native plant species has been suggested for the revegetation and the phytostabilization of the affected sites. Moreover, employing plant growth-promoting (PGP) bacteria to aid in phytoremediation processes is an effective technique that can enhance plant biomass production and stabilize metals. In a previous experimentation, we isolated the endophyte *Serratia* sp. strain RA59 from the hypogeal tissues of *P. lentiscus* and we characterized its PGP metabolic properties. Bioaugmentation assisted phytoremediation tests allowed us to demonstrate that the association between *P. lentiscus and Serratia* sp. RA59 is a promising option for metal phytostabilization programs in mining sites.

In this study, the physiological characterization of the strain and its ability to promote phytostabilization were deepened. *In vitro*, the strain tolerated high metal concentrations, mineralized soluble metal ions as detected by Environmental Scanning Electron Microscope, but it also solubilized insoluble forms of Cd and Zn. The strain RA59 was applied in a bioaugmentation assisted phytoremediation test under greenhouse conditions on metal contaminated substrate from the Iglesiente district. The plant survival and growth were monitored, the metal accumulation in hypogeal and epigeal parts was analysed, and the translocation factor was calculated. The obtained data were statistically analysed comparing: i) plants treated with the strain with the uninoculated control plants, ii) bioaugmentation-assisted phytostabilization by different plant species.

B7 Prebiotics, antibiotics and platform chemicals from enological by-products: a sustainable bio-economy perspective

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The wine sector is one of the pillars of Italian economy but also produces large amounts of by-products such as grape skins and stalks which are mostly disposed of for a fee. The present project aims to develop an integrated chemical-biotechnological process able to valorize all the components of this waste biomass: polyphenols are appreciated for their antioxidant and antimicrobial properties; cellulose can be fermented to lactic acid, the building block of the plastic biopolymers polylactides; hemicelluloses and pectins can be fermented to lactic acid or used as prebiotics.

Here, stalk fractions (polyphenols, cellulose, hemicellulose and pectins) were separated by enabling technologies, such as microwaves and ultrasound, in combination with subcritical water and a hydroalcoholic mixture, respectively, with no need of toxic solvents. Antioxidant activity of polyphenolic fraction was measured by different chemical methods (DPPH; ABTS; FRAP; Linoleic acid) and compared to common antioxidants (vitamin E and C). Furthermore, polyphenol antibiotic activity against *Pediococcus damnosus* and *Brettanomyces bruxellensis* (two wine contaminants) was tested. The cellulose fraction was fermented by engineered strains of *Clostridium thermocellum* with improved lactic acid production. In parallel, hemicellulose and pectin enriched-fractions were tested as enhancers of growth, aggregation, biofilm formation and adhesion of probiotic strains (*Enterococcus faecium* NCBI 10415 and *Lactobacillus acidophilus* CECT 4529) and enological starters (mainly *Saccharomyces cerevisiae*).

Through the results achieved, this study represents a proof-of-concept of a process leading to biorefining enological by-products.

B8 Engineered *Saccharomyces cerevisiae* for the upcycling of polyethylene terephthalate (PET) monomers

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Plastic has become an indispensable material in many fields, with production increasing every year; however, most of the plastic waste is still incinerated or landfilled and, only 10% of the new plastic is recycled even once. Among all plastics, PET is one of the most produced polyesters worldwide (56 Mt/year), and recently, its enzymatic hydrolysis has been proven at industrial level. Hence, the availability of new strategies for the convenient exploitation of PET-monomers is now increasingly relevant.

This work focuses on the development of new microbial routes for the upcycling of PET-derived monomers (terephthalic acid (TPA) and ethylene glycol (EG)) into industrially relevant organic acids (protocatechuic acid and glycolic acid). The combined use of the optimized synthetic biology tool (EASY-MISE toolkit)[1] and metabolic engineering approaches allowed us to create *Saccharomyces cerevisiae* strains harboring *ad-hoc* designed pathways for the above-mentioned bioconversions.

The outcomes that resulted from fruitful Design-Build-Test-Learn cycles will be here illustrated.

[1]Maestroni, L., Butti, P., Milanesi, R., Pagliari, S., Campone, L., Serra, I., Branduardi, Easy Modular Integrative fuSion-ready Expression (Easy-MISE) toolkit for fast engineering of heterologous productions in *Saccharomyces cerevisiae* (2023) ACS Synthetic Biology 12, 5, 1508–1519

https://doi.org/10.1021/acssynbio.3c00015

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B9 Modulation of sewage sludge microbiome for enhancing the transition from wastewater treatment plants into biorefineries in the circular economy era

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The sewage sludge (SS) microbiota operating in wastewater treatment plants (WWTPs) is determinant in pollutant degradation to obtain clean water. According to the WIDER-UPTAKE project [1], based on the circular economy principle of WWTP conversion into biorefineries, SS microorganisms can produce valuable materials, like polyhydroxyalkanoates (PHAs), using pollutants as nutrients [2]. Indeed, PHAs, produced as microbial intracellular storage compounds, are promising candidates as alternatives to petroleum-based plastics. PHAs can be efficiently produced by SS microorganisms through a process that involves the supplementation of volatile fatty acids (VFA) in specific PHA-producing reactors. Furthermore, VFAs can also be produced in specific WWTP fermenters by SS microorganisms through acidogenic fermentations. Therefore, studies on the relationships between operating conditions and SS microbiome can maximize VFA and, consequently, PHA production in a biorefinery system. In this study, different conditions were assayed at laboratory scale in batch-cultivation reactors and at pilot scale to improve VFA production by acidogenic fermentations, revealing microbiota structure changes by metataxonomics. In

particular, in batch-cultivation reactors, initial pH 10 determined methanogenic bacteria decrease and VFA producer increase [3], while the addition of $KMnO_4$ caused microbial diversity decrease and the acidogenic fermenting strain survival, thus increasing VFA yields. In pilot plant scale, the fermenter headspace reduction promoted VFA production, and comparative studies on SS microbiota structure are currently in progress. Thus, metataxonomics has been proven as a useful approach to highlight relationships between operating conditions, microbiomes, and the achievement of specific outputs.

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[3] https://doi.org/10.3390/microorganisms10102073

B10	***Bacillus subtilis* AC7 Fermentation on Rice Husk Substrate: A Sustainable Approach for Lipopeptide Biosurfactant Production**

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Biosurfactants are surface-active biomolecules synthesized by various microorganisms. Despite their diverse applications in environmental, industrial, pharmaceutical and biomedical fields, the high production costs limit their widespread use. To overcome this limitation, different renewable resources, particularly agro-industrial wastes, are being utilized as low-cost substrates for sustainable production. In this study, we explored the potential of the endophytic strain *Bacillus subtilis* AC7 in producing a lipopeptide biosurfactant using rice husks as a cost-effective renewable substrate. The production of lipopeptide AC7 was first optimized in batch. To determine the key factors influencing lipopeptide production, the Plackett-Burman design was employed incorporating various inorganic and organic compounds as supplements to the rice husk substrate. As a control, *B. subtilis* AC7 was cultivated in LB broth, following a method optimized for lipopeptide production. For each medium and condition, strain growth, surface tension of the cell-free supernatant, and crude extracts yield were evaluated. The highest yields were achieved by cultivating *Bacillus subtilis* AC7 in a 2.5% rice husk medium supplemented with NH_4NO_3 (3g/L), at 28°C for 48 hours, with continuous stirring at 140 rpm. MS and MS/MS analysis on the AC7 extract revealed that the qualitative composition of the lipopeptide obtained in the optimized rice husk medium was unchanged compared to that obtained in LB medium and consisted of a mixture of surfactin and fengycin. A semi-quantitative assay using LC-MS demonstrated that the AC7 lipopeptide in rice husk medium had a significantly higher yield than that obtained using rice husk alone, but a lower yield compared to LB medium. Ongoing investigations are focused on enhancing lipopeptide synthesis through the cultivation of *Bacillus subtilis* AC7 in laboratory-scale bioreactors. The use of process optimization strategies on rice husk and hydrolyzed rice husk is being explored to improve the efficiency and scalability of lipopeptide production.

B11	**Unlocking the potential of microbiome-based solutions as green biofertilizer for sustainable agriculture**

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The main objectives of the present work are to exploit the potential of specifically selected microbial consortia (MC)[1,2] for attaining sustainable agricultural production systems and to assess the impact of their application in field on indigenous rhizosphere microbial diversity. MC were applied alone or in combination with Arbuscular Mycorrhizal Fungi (AMF) and Biochar in open field under conventional and organic management and compared with commercial microbial products. The plant growth, diversity and composition of the maize rhizosphere microbiome and relative abundances of taxa were investigated at different maize growth stages and with different fertilization levels. The application of MC exerted a positive effect on plant growth especially at lower fertilization levels[3], while it did not significantly affect species diversity and richness of the native rhizosphere microbial communities. A great impact of biochar on rhizosphere soil microbiome was found suggesting that functionalization of biochar with MC seems a promising approach for microbiome modulation and enhancing plant growth[4]. WGS metagenome sequencing revealed slightly significant differences in community composition only between low and high fertilization levels and a significantly higher relative abundance of reads assigned to the SEED category “Nitrogen fixation” at low fertilization level (p-value of 0.03). Finally, genome sequencing of strains composing MC excluded any potential risk associated with scaling-up and their commercial application. Overall, our results suggest that multifunctional MC may be effectively exploited as biofertilizer in sustainable maize cultivation without altering the biodiversity or the resident microbiota, thus avoiding risks of long-term impacts on natural biodiversity.

Keywords: microbial inoculants, rhizosphere microbiome, whole metagenome seaquencing, sustainable agriculture

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B12 Assessment of the species-level infant gut bacterial biodiversity through a meta-analysis and the formulation of an optimized cultivation medium

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In vitro gut cultivation systems represent host-uncoupled, and cost-efficient approaches to evaluate the effects that intrinsic and extrinsic factors may exert on both composition and functionality of the gut microbiota. However, to guarantee the maintenance and survival of intestinal microbial players and preserve their functions, these *in vitro* models require close monitoring of several variables, encompassing pH, temperature, and oxygen concentration coupled with the use of a culture medium able to satisfy the expensive bacterial nutritional requirements. In this context, to identify the macro- and micro-nutrients necessary for *in vitro* cultivation of the infant gut microbiota, a meta-analysis based on 1669 publicly available shotgun metagenomic samples corresponding to fecal samples of healthy, full-term infants aged from a few days to three years was performed, allowing the identification of the predominant species characterizing the "infant-like" gut microbial ecosystem. Subsequently, infant fecal samples containing the identified most abundant bacterial taxa of the infant gut microbiota were cultivated on 18 different culture media and growth performances were evaluated through flow cytometry-based bacterial cell enumeration and shallow shotgun sequencing, allowing the formulation of an optimized growth medium, i.e., Infant Gut Super Medium (IGSM), which maintains and sustains the infant gut microbial biodiversity under *in vitro* growth conditions. Furthermore, this formulation was used to evaluate the *in vitro* effect of two drugs commonly used in pediatrics, i.e., acetaminophen and simethicone, on the taxonomic composition of the infant gut microbiota.

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Session C - Interactions between microbes/viruses and their hosts

C1 The influence of interferons on extracellular vesicles produced by primary monocyte-derived macrophages

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Cellular response to pathogens influences the production of cytokines, chemokines and Extracellular Vesicles (EVs). Interferons (IFNs) are fundamental effectors of antimicrobial innate immunity and important regulators of the adaptive immune response. Aim of this work was to analyze whether type I (IFNα2b and IFNβ), II (IFNγ) and III (IFNλ) IFNs can influence the production and composition of the EVs. As cellular model we used primary monocyte-derived macrophages (MDM) of healthy donors differentiated using GM-CSF (Granulocyte-Macrophage-Colony-Stimulating-Factor). Small-EVs (sEVs) were purified by size exclusion chromatography (SEC) from supernatants of cell treated for 20 hours. To characterize concentration and dimensions, the vesicles were analyzed with Nanoparticle Tracking Analysis (NTA). SEVs surface markers were examined by a new flow cytometric multiplex bead-based platform (MacsPlex exosomes human kit) to evaluate the expression level of 37 different membrane proteins. Although no significant changes in the number or size of sEVs were observed, IFNs treatments induces a significant down-regulation of CD9, CD63, CD81 exosomal markers on sEVs. In addition, IFN-EVs showed a significant modulation of some adhesion molecules, co-stimulatory protein and of major histocompatibility complexes suggesting sEVs participate in IFNs mediate cellular response. Experiments are in progress using autologous PBL treatments with MDM-IFNs-sEVs to evaluate their impact on T and B lymphocyte response. Altogether, our results show that EVs are affected by the IFNs treatments, that can alter their “message” and might induce different outcomes in target cells.

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C2 Organelle remodeling by positive-sense single-stranded RNA viruses

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Positive-sense single-stranded RNA (+RNA) viruses infection induces the formation of viral replication organelle (RO), a specialized membrane-delimited organelle where the viral genome replication takes place. SARS-CoV-2 ROs are composed of double membrane vesicles that originate from and are in contact with the endoplasmic reticulum. Electron tomography coupled with light microscopy showed how viral infection reshapes all cellular organelles to create an environment conducive to viral infection. To date, the host and viral factors and the molecular mechanisms that bring to the formation of the viral ROs and are responsible for the cellular alterations induced by SARS-CoV-2 are still unknown. By integrating imaging and organelle proteomics we have identified potential host factors co-opted by SARS-CoV-2 for its replication. Those factors can be used as future targets for developing novel antivirals that might help to curb future pandemics.

C3 Microbial genetic strategies for sex-specific gut persistence throughout the human life

C. Tarracchini[1], G. Alessandri[1], F. Fontana[1,2], S.M. Rizzo[1], G.A. Lugli[1], M.G. Bianchi[3], L. Mancabelli[1], G. Longhi[1,2], C. Argentini[1], L.M. Vergna[1], R. Anzalone[2], A. Viappiani[2], F. Turroni[1,4], G. Taurino[3], M. Chiu[3], S. Arboleya[5], M. Gueimonde[5], O. Bussolati[3,4], D. van Sinderen[6], C. Milani[1,4], M. Ventura[1,4]

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While the compositional variation of the gut microbiome during human development has been extensively investigated, the strain-resolved changes remain largely unexplored. In the current study, metagenomic sequencing data of 12,415 fecal microbiomes from healthy individuals were employed for strain-level tracking of gut microbiota members to elucidate colonization and persistence patterns across the human life span. This analysis revealed host sex-related persistence of strains belonging to commonly maternally-inherited species, such as *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *longum*. Comparative genome analyses showed that specific microbial glycosyl hydrolases involved in host-glycan metabolism may contribute to more efficient colonization in females compared to males. These findings point to an ancient host-microbe co-evolution process driving the persistence during the reproductive age of females of key microbial taxa known to be frequently transmitted from mothers to newborns.

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C4 *Bacillus* spores exert a protective and immunomodulatory activity in mice with DSS-induced colitis

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Keywords: *Bacillus subtilis*, *Alkalihalobacillus clausii*, spores, gut, inflammation

Spore-forming bacteria of the *Bacillus* genus are widely used in various probiotic formulations, due to their beneficial properties, improvement of intestinal barrier function, antimicrobial and immunomodulatory activity. In the animal gut, both spores and germination-derived cells promote the development of Gut-Associated Lymphoid Tissue (GALT), exert immunomodulatory properties and reduce the symptoms of intestinal inflammation. Two strains isolated from healthy human biopsies, *Bacillus subtilis* SF106 and *Alkalihalobacillus clausii* SF174, have been previously characterized and proposed as probiotics. The oral administration of these spores in a murine model of DSS (Dextran-Sulfate-Sodium)-induced colitis appears to mitigate intestinal inflammation

and promote intestinal barrier integrity, as observed by significant overexpression of the *muc-2* gene (for mucin production) and the *claud-1* gene (for tight junctions). This was confirmed by histological analysis, which showed that the intestinal epithelial structure was restored in both spore-treated groups of mice. Colon sections also showed a general reduction in inflammation, with reduced gene expression of the pro-inflammatory cytokine TNF-α, while no significant variation was observed for the expression levels of IL-1β and IL-6. Interestingly, gene expression level of the anti-inflammatory cytokine IL-10 was found to be significantly higher in mice treated with SF174 spores. Different expression of the Toll-like receptors TLR-2 and TLR-4, which are known to be involved in anti-inflammatory and pro-inflammatory signaling pathways, was also found. Gut microbiota analyses showed that both spore types increased gut microbial diversity and had a positive effect on the abundance of beneficial bacteria.

C5 LysX2 is a *Mycobacterium tuberculosis* membrane protein with an extracytoplasmic MprF-like domain

D. Sorze[1], F. Boldrin[1], L. Cioetto Mazzabò[1], M.A. Lanéelle[3], L. Rindi[4], G. Segafreddo[1], A. Lemassu[3], G. Etienne[3], M. Conflitti[1], S. Sultan[2], M. Daffé[3], A. Garzino Demo[1,5], R. Manganelli[1], H. Marrakchi[3], R. Provvedi[2]

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Using a combination of bioinformatic, genetic and bacteriological methods, we characterized a protein of *Mycobacterium tuberculosis(Mtb)*, Rv1619 (named LysX2), carrying a domain with high similarity to aminoacyl-phosphatidylglycerol synthases belonging to the family of MprF-like proteins. However, unlike homologous domains of MprF of *S. aureus* and LysX of *Mtb*, that are positioned in the cytoplasm, we predicted that the MprF-like domain in LysX2 is in the extracytoplasmic region.

Moreover, we found LysX2 orthologues in major human pathogens and in rapid-growing mycobacteria frequently associated with human infections, but not in environmental and non-pathogenic mycobacteria.

Expression of *lysX2* in *Mycobacterium smegmatis* increased cell resistance to human β-defensin 2 and sodium nitrite, enhanced cell viability and delayed biofilm formation in acidic pH environment. LysX2 significantly reduced the negative charge on the bacterial surface upon exposure to an acidic environment.

These data reveal a potential role of LysX2 in the response to acidic pH: we started investigating its function in the adaptation to this environment in *Mtb*. We observed that the *lysX2*-knock-out strain exhibited significantly enhanced growth on an acidified medium compared to wild type *Mtb*, revealing a potential role of LysX2 in sensing the pH of the environment and contextually in modulating the growth rate. Because LysX2 is present only in pathogenic mycobacteria and this phenotype has not been observed in the non-pathogenic species *M. smegmatis*, we hypothesize that LysX2-driven adaptation to pH may be critical to *Mtb* pathogenesis.

C6 The Zn/Cd efflux systems of *Salmonella* Typhimurium are critical for the colonization of plants

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Enteric human pathogens such as *Salmonella* Typhimurium (STM) can enter, replicate and persist inside plant tissues for several days, making raw edible plants possible pathogen reservoirs. The versatility of STM in the colonization of different niches also depends on its ability to respond efficiently to fluctuations in micronutrient availability. Zinc plays an essential role in bacterial physiology, and its concentration influences bacterial survival. Although it is well established that animals employ zinc nutritional immunity strategies to fight pathogens, it is unknown if this metal plays a role in the outcome of STM-plant interaction. In this study, we have investigated the involvement of STM Zn/Cd detoxification systems in plant colonization, using *Arabidopsis thaliana* as a model host.

Our results show that, during plant colonization, STM faces high zinc concentrations and takes advantage of its ability to export excess metal through the efflux pumps ZntA and ZitB. Deleting the Zn/Cd efflux systems affects bacterial persistence in the shoots to an extent that depends on metal availability in the plant tissues., Interestingly, we also show that plants increase the expression of specific root-to-shoot Zn transporter and translocate this metal to the site of infection as a response to STM colonization.

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We thus provide preliminary evidence that plants respond to enteric pathogens by nutritional immunity mechanisms involving Zn intoxication. Our work highlights the role of Zn in STM-plant interaction and suggests that modulation of plant metal content through biofortification may be an efficient strategy to control enteric pathogen colonization.

C7 Characterisation and immunomodulatory potential of extracellular vesicles from non-pathogenic yeast strains isolated from a fermented product

S. Nenciarini[1], R. Amoriello[2], G. Bacci[1], B. Cerasuolo[1], M. Di Paola[1], P. Nardini[2], M. Pivetti[1], S. Mucci[1], A. Papini[1], C. Ballerini[2], D. Cavalieri[1]

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Extracellular vesicles (EVs) are lipid-bilayered particles, containing various biomolecules, including nucleic acids, lipids and proteins, released by cells from all the domains of life. They perform multiple communication functions, exerting them either intra- or inter-kingdom. Evidence suggests that the interaction between host immune cells and small RNAs carried by fungal EVs induce modulation of the host immune system. To date, most of the studies on fungal EVs immunomodulation have been conducted in the context of fungal infections, therefore there is still a knowledge gap regarding EVs produced by non-pathogenic yeasts. In this work, we characterised EVs obtained by Saccharomyces cerevisiae and Pichia fermentans strains isolated from a fermented milk product with probiotic properties. Immunomodulation abilities of EVs produced by these strains have been studied in vitro through immune assays after internalisation from human monocyte-derived dendritic cells. Results showed a significant reduction of antigen presentation activity of dendritic cells treated with EVs derived by yeast strains from the fermented milk. The

small RNA fraction of EVs contained a vast majority of yeast mRNA sequences with molecular functions shared between strains of different species isolated from the same matrix. Our results suggest that one of the mechanisms behind the anti-inflammatory properties of probiotic foods could be mediated by the interactions of human immune cells with yeast EVs. These insights are preliminaries to further investigations on clinical applications of fungal EVs as suitable candidates for immunomodulatory therapy delivery in several human conditions.

C8 Engraftment of Viable Microbiota after Fecal Transplantation Drives *Clostridiodes difficile* Inhibition

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Despite the efficiency of fecal microbiota transplantation (FMT) to treat recurrent *Clostridiodes difficile* infection (CDI), its mechanisms of action remain poorly understood. While microbiome engraftment post-FMT is thought to drive the inhibition of *C. difficile*, studies to date have only used sequencing-based methods, which cannot distinguish cell viability nor quantify bacterial load of patients. Here, we combine high-throughput sequencing with Fluorescent Activate Cell sorting (FACS) to characterize and quantify the viable microbiome and its function before and after transplantation. We demonstrate that FMT does not restore bacterial viability in patients, which remains lower than in healthy donors, and that engraftment is dependent on pre-FMT bacterial load. Further, we show that primary and secondary bile acids, butanoate, propanoate, sphingolipids, sugar amino and nucleotide sugar metabolism bacterial pathways, related to inhibition of *C. difficile*, are restored when using absolute quantification, but not when using relative abundances derived from sequencing alone, and identify a subset of 6 transferred viable bacteria genera, such as *Bifidobacterium spp.*, *Parabacteroides spp.*, *Collinsella spp*, *Bacteroides spp.*, *Dorea spp.* and *Roseburia sp.p*, that contribute to these functions. Our findings demonstrate that quantification and viability are fundamental parameters to accurately characterize the microbiome in FMT, and provide novel bacterial targets to refine this therapeutic procedure.

C9 Role of the MDR Efflux Pump AcrAB in Epithelial Cell Invasion by Shigella flexneri

M. Coluccia, A. Béranger, R. Trirocco, G. Fanelli, F. Zanzi, B. Colonna, M. Grossi, G. Prosseda, M. Pasqua

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The tripartite complex AcrAB-TolC is the major RND pump in Escherichia coli and other Enterobacteriaceae, including Shigella, the etiological agent of bacillary dysentery. In addition to conferring resistance to many classes of antibiotics, AcrAB plays a role in the pathogenesis and virulence of several bacterial pathogens. Here, we report data demonstrating that AcrAB specifically contributes to Shigella flexneri invasion of epithelial cells. We found that deletion of both *acrA* and *acrB* genes causes reduced survival of S. flexneri M90T strain within Caco-2 epithelial cells and prevents cell-to-cell spread of the bacteria. Infections with single deletion mutant strains indicate that both AcrA and AcrB favour the viability of the intracellular bacteria. Finally, we were able to further confirm the requirement of the AcrB transporter activity for intraepithelial survival by using a specific EP inhibitor. Overall, the data from the present study expand the role of the AcrAB pump to an important human pathogen, such as Shigella, and add insights into the mechanism governing the Shigella infection process.

Coluccia, M., Béranger, A., Trirocco, R., Fanelli, G., Zanzi, F., Colonna, B., Grossi, M., Prosseda, G., & Pasqua, M. (2023). Role of the MDR Efflux Pump AcrAB in Epithelial Cell Invasion by Shigella flexneri. Biomolecules, 13(5), 823. https://doi.org/10.3390/biom13050823

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C10 Identification of new benzofuran derivatives as STING agonists with broad-spectrum antiviral activity

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STING is a transmembrane protein localized in the endoplasmic reticulum involved in type I Interferons (INF-I) transcription. When cytosolic DNA is detected cyclic GMP-AMP (cGAMP) synthase produces 2'3' cGAMP, that binds STING triggering IFN Regulatory Factor 3 (IRF3) phosphorylation and dimerization, this complex is translocated into the nucleus where stimulates IFN-I transcription. Recent studies identified STING agonists with antiviral activity.

For this reason, a selection of putative STING-ligands was tested to evaluate their ability to induce IFN-I and inhibit viral replication. By applying two rounds of similarity ligand-based virtual screenings a focused library of eleven benzofurans has been selected and subjected to biological test. A gene reporter assay in cells expressing exogenous STING (HEK293T) was used to investigate compounds' dependent IFN-b transcription in presence of a luciferase reporter gene driven by the human IFN-b promoter. Tested compounds induced IFN-b transcription, while they were not able to induce IFN-b transcription in presence of mutated and inactive STING suggesting a specific protein-ligand interaction. The best hit compounds were tested for the antiviral activity in BEAS-2B cells against human coronavirus 229E: one of them showed antiviral activity whit an EC50 value in the μM range. The same compound was tested to evaluate its antiviral effect

against SARS-CoV2 in BEAS-2B cells and Vero E6. Since DNA damage can indirectly activate STING and parallel pathways inducing IFN response, we evaluated compound's mediated genotoxic effect, through p53 levels analysis, as well as dihydroorotate dehydrogenase inhibition showing no effects in both cases.

C11	**Genetically Modified M13 Bacteriophages for Precise Photodynamic Cancer Eradication**

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Photodynamic therapy (PDT) is a promising approach for cancer treatment. This study aimed to enhance PDT's effectiveness by engineering M13 bacteriophages as targeted vectors for efficient photodynamic killing of cancer cells. M13 phages were genetically engineered to display the 7D12 nanobody, enabling specific recognition of the Epidermal Growth Factor Receptor (EGFR), overexpressed in various cancers with severe outcome.

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The modified phages $M13_{EGFR}$, were chemically conjugated with fluorescent molecules and Rose Bengal (RB) photosensitizers on the capsid surface. The study demonstrated that the engineered $M13_{EGFR}$ selectively targeted $EGFR^+$ A431 cell line while showing minimal binding to an EGFR-negative control line.

Therapeutic performance of the modified phages was evaluated in both 2D and 3D cell culture models. Notably, in 3D spheroid models, the phages successfully penetrated the spheroid core, indicating their potential to target and treat cancer cells within complex tumor microenvironments. Remarkably, these modified phages exhibited potent and selective photodynamic killing activity at nanomolar concentration of RB and picomolar concentration of phages upon irradiation with a white LED lamp.

To assess translational potential, a yellow LED laser with higher irradiance was incorporated into an in vivo setting. Preliminary results demonstrated promising outcomes, suggesting future clinical applications of this technology. Further in vivo investigations on murine models (xenografts) are ongoing.

We successfully engineered M13 bacteriophages as targeted vectors for enhanced photodynamic therapy and we demonstrated specific binding, potent precision photodynamic killing activity and the ability to penetrate tumor spheroids. These findings hold promise for the development of a highly efficient and targeted approach to eradicate cancer cells.

C12 Fitting the best statistical model: A case study on longitudinal urinalysis data of uropathogenic *Escherichia coli* infection in mice

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Statistical analysis is integral to mouse infection studies and typically endeavours to explain infection phenomena from explanatory variables. A linear regression model is often employed, which is essentially an optimisation procedure with the incorporation of uncertainty. To obtain robust estimates of the uncertainty, assumptions such as normality, linearity and homoscedasity apply. Here we describe the process of arriving at the most appropriate regression model, using longitudinal urinalysis data (viable bacterial counts in urine) from mice with experimental urinary tract infection induced by uropathogenic *Escherichia coli* (UPEC). Groups of wild-type C57BL/6 (n=26) and *rag1*$^{-/-}$ mice (n = 11) defective in adaptive immune responses, were inoculated with UPEC in the bladder and colony forming units (CFU) in urine were measured over 28 days. Data were analysed using a series of statistical models for parsimony and appropriateness, ranging from a simple regression to mixed models, additive mixed models, and zero-inflated negative binomial mixed models (ZINBMM). The best fitted model was the ZINBMM, which provided an elegant way to answer to two questions: (i) “what is the probability that each strain of mice will be colonised?” and (ii) “once colonised, is there a difference between the two mouse strains with respect to bacterial burden?”. The ZINBMM uncovered a difference in the colonisation probability between mouse strains, but once infection was established, both maintained similar urinalysis profiles. Our findings support a role for adaptive immunity in urinary tract infection control in mice and demonstrate that finding the best-fit statistical model can meaningfully explain biological processes.

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POSTER SESSIONS

Session A - Microbial genetics and genomics

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A13 New insights on the regulation of the biosynthesis of the glycopeptide antibiotic A40926 by *Nonomuraea gerenzanensis*

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Nonomuraea gerenzanensis is an actinomycete of industrial interest for its ability to produce the glycopeptide antibiotic A40926, the precursor of the antibiotic dalbavancin, in clinical use to treat skin infections due to multi-drug resistant Gram-positive bacteria. The biosynthetic gene cluster of the glycopeptide A40926 (*dbv* cluster) contains protein-coding sequences responsible for antibiotic biosynthesis, regulation, resistance, and export. A40926 biosynthesis is positively controlled by two pathway-specific regulators, Dbv4 (StrR-like) and Dbv3 (LuxR-like), and negatively modulated by the two-component system, constituted by the response regulator Dbv6 and the sensor-kinase Dbv22.

To get more insights into the physiology and antibiotic biosynthesis, the characterization of a spontaneous mutant and targeted mutants is under investigation.

The spontaneous mutant was obtained after repetitive cultivation in the presence of high concentrations of apramycin and features impairments in growth and pigmentation. The analysis of its genomic sequence compared to the parental strain is ongoing to explain these phenotypic differences. The targeted mutant is a strain containing an inducible copy of *iclR*, the isocitrate lyase regulator in *Escherichia coli*. This protein was recovered from *N. gerenzanensis* crude cell lysates through a pulldown assay using the upstream region of *dbv4*. Transcriptional, morpho-physiological, and chemical analyses, by HPLC-MS, of the over-*iclR* strain revealed that IclR negatively controls the *dbv4* transcription inhibiting the A40926 production. The construction and characterization of an in-frame *iclR* deletion mutant strain are ongoing.

Besides leading to improved glycopeptide-producing strains, the results of this work may expand our knowledge on the physiology and glycopeptide biosynthesis in actinomycetes.

A14 HGFiT: Assessing the impact of HGT on metabolic fitness through the simulation of 11 million transfer events

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Horizontal gene transfer (HGT) is the acquisition of genetic material outside of a parent-offspring relationship. While it has been detected across all domains of life, its relevance in bacteria is of critical concern if we think of the role it plays in, for example, antimicrobial resistance. From a basic perspective, a horizontally acquired gene may have a positive or negative impact on the bacterium's fitness, depending on its position in the recipient's molecular circuitry, and on the baseline energetic cost on a cell via the demands at the DNA, RNA, and protein levels that it imposes. Despite being one of the drivers of evolution, to this day, a large-scale computational model of the effect of HGT on bacterial metabolic fitness is lacking.

How does an HGT event affect metabolic fitness in the light of this demand/benefit trade-off? Here we developed a computational pipeline to simulate the random transfer of DNA fragments among genomes and estimate their (positive or negative) impact on microbial metabolic phenotypes using constraint-based modelling. We simulated 11,121,011 transfers of DNA fragments from 4467 species across Bacteria and Archaea domains (donors) to *E. coli* (recipient) and evaluate the impact on the recipient's metabolic fitness. By doing so, we identified trends (involving donor-recipient phylogenetic distance, length of the transferred fragment, etc.) that illuminate on the role of HGT in microbial evolution and on the factors that influence it.

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A15 Therapeutic potential of tradeoffs during adaptation of *E. coli* to the inflamed mouse gut

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Inflammatory bowel disease is characterized by changes in the gut, and IBD patients are known to show gut dysbiosis when compared to healthy individuals. Disease-mediated changes in the intestine impose different selection pressures on the microbiome, resulting in selection for disease-specific microbial traits.

In our study, we performed a long term in-vivo evolution experiment of *Escherichia coli* in a mouse model of IBD to study the adaptation of the gut microbiome to chronic inflammation within a host's lifetime. Germ-free mice (WT vs. IL10-KO) were monocolonised with a single isolate of *E. coli* that was allowed to adapt to the mouse gut for three months. Fecal samples were analyzed by using a multi-omics approach. Shotgun metagenomics, metabolomics and Biolog plates were applied to identify mutations, changes in the gut metabolites and the metabolic repertoire of the evolved populations. We identified a locus in the *E. coli* genome that may be involved with resistance to oxidative stress, that is associated to populations evolved in the inflamed gut and disease-specific bacterial traits that evolve in *E. coli* during adaptation to the chronically inflamed murine intestine. Notably, the evolution of such disease-specific traits is accompanied by tradeoffs that may be suitable for exploiting clinically.

A16 Targeting *Helicobacter pylori* transcriptional regulator HP1043 as a novel antimicrobial approach

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Antibiotic-resistant bacteria are a major threat to public health. *Helicobacter pylori* is a successful human pathogen that colonizes stomach epithelium and rapidly develops resistance to standard therapies, drastically reducing its eradication rate. Among the only two *H. pylori* orphan response regulators, HP1043 is a pivotal transcription regulator (TR) that controls essential cellular processes. Consequently, the *hp1043* gene cannot be deleted, nor can the amount of protein be modulated. This aspect poses challenges for researchers and, simultaneously, makes it a good candidate for novel antimicrobial therapies. We designed a reporter system to investigate *in vitro* and *in vivo* HP1043 regulatory activity on validated target genes. Afterwards, we examined the correlation between the HP1043 consensus sequence and the -10 element mutual distance concerning the transcriptional regulation of the protein. To further study the potentiality as a novel therapeutic target, we employed antisense short oligomers, called peptide nucleic acids (PNAs), to modulate the HP1043 protein level. In virtue of their gene knockdown capability, specific PNAs were designed to silence *hp1043* mRNA. These short oligomers can be easily functionalized and redesigned to enhance the antisense efficacy and ensure cell internalization. Targeting a TR that controls a cluster of fundamental genes is the first example of a specific multitargeting approach against H. pylori. Additionally, the use of PNAs can be a versatile tool to deeply understand the regulatory mechanism of other TRs whose study is hampered by their crucial role in the cell.

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A17 The core genome evolution of *Lactobacillus crispatus* as a driving force for niche competition in the human vaginal tract.

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It has been widely reported that members of the genus *Lactobacillus* dominate the vaginal microbiota, which is represented by the most prevalent species, *i.e.*, *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri* and *Lactobacillus iners*. Between them, *L. crispatus* is considered an important microbial biomarker due to its professed beneficial implications on vaginal health. In order to identify molecular mechanisms responsible for health-promoting activities that are believed to be elicited by *L. crispatus*, we performed *in silico* investigations of the intraspecies biodiversity of vaginal microbiomes followed by *in vitro* experiments involving various *L. crispatus* strains. In this study, we evaluated the single nucleotide variation within protein-encoding genes shared across a *L. crispatus* strain selection, which includes 200 currently

available human-derived *L. crispatus* genomes as well as 41 newly sequenced chromosome of such taxon. Particularly, two single nucleotide variations in the type II pullulanase gene sequence led to specific amino acid substitutions, possibly explaining the substantial differences in the growth performances and competition abilities observed in a multi-strain bioreactor culture simulating the vaginal environment. This study pointed out the presence of intra-species micro-diversities that could have evolutionary significance contributing to phenotypical diversification by affecting protein domains.

A18 Genomics of *Acinetobacter baumannii* iron uptake

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Iron is essential for the growth of nearly all bacteria due to its redox activity and its role in many vital metabolic reactions. The nosocomial pathogen *Acinetobacter baumannii*, responds to low iron availability imposed by the host through the exploitation of multiple iron-acquisition strategies, which deliver iron to the cell under different environmental conditions, including human and animal infection. Currently, six different gene clusters for active iron uptake have been described in *A. baumannii*, encoding protein systems involved in: *i*) ferrous iron uptake (*feo*); *ii*) heme-uptake (*hemT* and *hemO*), and *iii*) synthesis and transport of the baumannoferrin (*bfn*), acinetobactin (*bas/bau*) and fimsbactin(s) (*fbs*) siderophores. Several clonal lineages of *A. baumannii*, including ST2, ST1, ST79, and ST25, are responsible for most hospital outbreaks worldwide. We investigated the structure, distribution, and phylogeny of iron uptake gene clusters among > 1,000 genotypically diverse *A. baumannii* isolates to determine any correlation between the emergence of certain epidemic clones and their ability to overcome the extreme iron limitation imposed by the host. We found that *feo*, *hemT*, *bfn* and *bas/bau* clusters are very prevalent across *A. baumannii* clones (>98% of isolates), whereas the additional heme uptake system *hemO* is present in only a portion of the isolates (69%), and the *fbs* gene cluster is very rare (1%). Since the expression of multiple iron uptake clusters can be linked to infectivity and virulence, the presence of the additional heme uptake system *hemO* may have contributed to the spread of some clones belonging to the most successful *A. baumannii* lineages.

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A19 Interplay of CodY and CovR in regulating gene expression in Group B *Streptococcus*

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Group B *Streptococcus* (GBS) can switch from a harmless commensal of the urogenital tract of healthy adults, to an invasive pathogen responsible for severe infections affecting newborns, pregnant women and fragile individuals. This transition is initiated by still undefined environmental signals that activate gene regulatory pathways promoting rapid adaptation to different host niches. Although a few conserved two-component systems and transcriptional regulators have been described, information on the mechanisms allowing integration of these regulatory systems is lacking.

We recently identified CodY as a global transcriptional regulator required for virulence in GBS. Comparative transcriptomic analysis highlighted an extensive overlap between the CodY regulon and genes directly controlled by the master regulator of virulence CovR.

With the aim to uncover the interplay between CovR and CodY in GBS, here we determined their relative contribution in the control of a selected set of shared targets. Derivatives of a hypervirulent GBS strain carrying the marker-less deletion of *codY*, *covR* or of both genes were prepared. Electrophoretic mobility shift assays and beta galactosidase activity assays with wild-type and mutated *lacZ* transcriptional fusions were performed to dissect the mechanism of gene regulation played by each factor.

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Despite the interplay between CodY and other transcriptional regulators was previously reported in several organisms, the results here obtained suggest a novel mechanism by which CodY and CovR cooperate in GBS. In this bacterium, these two regulators do not control each other's transcription but their activity is strictly interlinked, with CodY being required for CovR-mediated regulation of gene expression.

A20 Application of the *Escherichia coli* model system to the study of human polyribonucleotide phosphorylase

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Polyribonucleotide phosphorylase (PNPase) is a phosphorolytic RNA exonuclease highly conserved from bacteria to human. In mammals, PNPase (hPNPase) is located in mitochondria, and it has been implicated in RNA import from cytoplasm, in mitochondrial RNA degradation and in the processing of R-loops, namely stable RNA-DNA hybrids displacing a DNA strand. Mutations in the hPNPase *PNPT1* gene cause severe neurological diseases ranging from hereditary hearing loss to multisystem oxidative phosphorylation deficiency disorders and Leigh syndrome. To correlate specific mutations with the protein molecular defects and the clinical symptoms is not an easy task given the plethora of processes involving hPNPase and the small number and heterogenous genetic background of patients. We are exploiting *Escherichia coli* strains expressing hPNPase variants as a tool to characterize the molecular activity of disease-linked hPNPase

variants. To this aim, we constructed a chimeric *E. coli* strain expressing hPNPase and studied its phenotype. We found that hPNPase modulates, directly and/or indirectly, around 200 bacterial genes. Interestingly, the SOS response, which is induced by DNA damage, is constitutively active in presence of hPNPase. We identified two hPNPase-dependent mechanisms that can explain SOS induction in *E. coli*, namely oxidative stress generation and R-loops accumulation induced by hPNPase. We are currently analysing the impact of four hPNPase mutations causing different pathologies on the chimeric strain phenotype.

A21 Core genome and essential gene burden impacting on fitness costs of the extensive-last resort antimicrobial-resistance in CA-MRSA genome

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Background

Antimicrobial-Resistance (AMR) can determine variable Fitness Costs (FCs) (growth-rate, competitive growth-ability, virulence). FC key-mechanisms are poorly characterized, however associable to mutations or alteration in vital-pathways of adaptable genomes. Newly, we discovered an adaptive glycopeptide-reduced-susceptibility in CA-DAP^{R}-GISA paying high-FCs. Understanding the FC basis of extensive-drug-resistance in prone genomes is crucial for predicting the potentiality of the AMR onset/prevalence/co-occurrence.

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Our goal is to investigate the core-genome (cg) and essential gene (EGs) burden in CA-MRSA genome with daptomycin and adaptive glycopeptide reduced-susceptibility showing high-FCs.

Methods

CA-DAP^{R}-GISA versus its CA-DAP^{S}-GSSA parent comparative genomics and transcriptomics were investigated by Illumina-Miseq Whole-Genome and RNA Sequencing. cgSNPs were predicted by Snippy-core, and SNP-effects by snpEff on MW2 RefGen. EG-Transcriptomics were investigated by Rockhopper, DAVID, Gene-Ontology and KEGG.

Results

Comparative core-genomics evidenced 8 cgSNPs with 7 in EGs. Moderate-Impact SNPs were found in EG i.e., rpoB (transcription and RIF-R), murG and mprF (cell-envelope and the last also DAP-R), gdpP (β-lactam resistance). 3 SNP-reversions were in EG tagU and glnA (teichoic acid and glutamine biosynthesis), alongside recU (DNA-packaging). Comparative transcriptomics showed 46 under- *vs* 36 over-expressed EG-mRNAs whilst 56 under- *vs* 25 over-expressed EG small-antisense RNAs (asRNAs). Key under-expression was in Protein-Synthesis and Cell-Envelope EG-clusters, followed by the DNA- and RNA-metabolism ones. Key dysregulation, *via* asRNAs, was in cell-envelope, DNA-RNA-metabolism, and cofactor EG-pools.

Conclusions

Our outputs recognised the Protein-Synthesis, Cell-Envelope and DNA-RNA-metabolism vital pathways as core-genome and EG “hot-spots” of omic-events related to the extensive-last resort antimicrobial -resistance FCs in CA-MRSA showing a high-genomic plasticity and complex regulatory-interactions shaping gene-expression.

A22 Pirin, a redox-sensitive modulator of beta-oxidation, exploits flavonoids to manage oxidative stress in bacteria

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The Pirin family includes proteins conserved throughout evolution, from bacteria to humans. In prokaryotes, these proteins are present in many taxonomic groups and can be present in multiple copies. Only a few of these proteins have been studied. In *Serratia marcescens*, a protein of the Pirin family is an inhibitor of pyruvate dehydrogenase while the YhhW pirin of *Escherichia coli* has quercetinase activity. In *Streptomyces ambofaciens* PirA plays an important role in cellular metabolism and oxidative stress. Inactivation of *pirA* resulted in marked effects on central carbon and energy metabolism, high sensitivity to H_2O_2, repression of polyketide antibiotic production, and substantial accumulation of total lipid esters in triacylglycerol stores. Most of these effects are due to the inability of the *pirA*-defective strain to modulate the beta-oxidation pathway. Indeed, PirA interacts with very long-chain acyl-CoA dehydrogenase AcdB, which catalyzes the first committed step of the beta-oxidation pathway and is a redox-sensitive negative modulator of its activity. In the present study, we demonstrate that inactivation of *pirA* results in an increase in the endogenous production of H_2O_2, and that *Streptomyces* PirA, similar to *E. coli* YhhW Pirin and human hPirin, has quercetinase activity. Furthermore, we show that, by interacting with AcdB and oxidizing quercetin, PirA attenuates the oxidative injury due to H_2O_2 that accumulates during the beta-oxidation as a consequence of the AcdB-catalyzed reaction if the electron transport chain is saturated. Since Pirins are evolutionarily conserved in all the kingdoms of life, this mechanism may extend to other organisms as well.

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A23 Characterization of Multidrug Resistance *Klebsiella pneumoniae* strains from *Lesser kestrels*

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Antimicrobial Resistance (AR) and the progressive isolation of multidrug resistant bacteria (MDR) from clinical and environmental samples, have been recognised as a global public health problem. The upsurge of AR is mainly due to both horizontal transfer, mostly mediated by conjugative plasmids (CPs), of antimicrobial resistance genes (ARGs) and the spread of MDR clonal strains. Within this framework wild animals have recently been found to play a non-secondary role. In this study we report preliminary data on detection and characterisation of MDR *Klebsiella pneumoniae* strains (KP) isolated in Apulia in 2021 and 2022 from *Lesser kestrels*. Clonal relatedness assessed by pulsed-field gel electrophoresis (PFGE), established the presence of two distinct and clonally unrelated groups of strains: the 2021 and 2022.

The 2021 strains harboured a conjugative (frequency of $1x10^{-6}$ donors/transconjugants) IncFIIk/IncFI-B plasmid (223.558 bp). The conjugative plasmid included two regions of which one carried genes encoding resistance to heavy metals (As, Ag and Cu) and an iron uptake system; the second ARGs (*aac(3)-IIa, aac(6')Ib-cr, strAB*), (*bla*$_{CTX-M-15}$, *bla*$_{TEM1B}$, *bla*$_{OXA-1}$), (*qnrB1*), (*dfrA14,sul2*) and (*tetA*), encoding resistance to aminoglycosides, beta-lactams, quinolone, trimethoprim-

sulfamethoxazole and tetracycline, respectively.

Insertion sequence elements of different classes were scattered along the two regions. Noteworthy was the presence of eight IS26 clustered within ARGs.

This preliminary study has highlighted for the first time the potential contribution of *L. kestrels* in the spread of AR. Besides, it poses open questions such as the acquisition of a MDR-CP mainly detected in KP isolated from clinical cases.

A24	**Covid-19 genomic surveillance in Bangui (Central African Republic) reveals a landscape of a circulating variant linked to validated antiviral targets of SARS-CoV-2 proteome**

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Since its outbreak at the end of 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly causing coronavirus disease 19 (Covid-19) pandemic. Even with the administration of a high number of vaccine doses, the virus continued to transmit among people because of inequal access to those therapeutics and of differences in containment measures. Information about COVID-19 in the African continent have been poor and contradictory, and even regional studies are important. On these premises, we conducted a genomic surveillance study about COVID19 lineages circulating in the area of Bangui, capital of the Central African Republic (CAR). We collected 2,687 nasopharyngeal samples at four checkpoints in Bangui, in the period spanning from July 2nd to 22nd, 2021. Fifty-three samples tested positive to SARS-CoV-2 and were sequenced for the presence of different viral strains. We performed phylogenetic analysis and described the lineage landscape of SARS-CoV-2 circulating in CAR in the study period, finding the Delta variant as the major lineage. We thoroughly analysed natural mutations at genes encoding for validated antiviral targets such as non-structural protein (Nsp) 3, 5, 7, 8, 12, (nucleoprotein) N, and (spike) S. The results showed that mutations were both defining and non-defining the Delta variant, which was arising at the time of the survey as variant of concern (VOC). Despite the limited number of positive samples detected, this study provides valuable information of COVID19 evolution at the regional level and allows a better understanding of SARS-CoV-2 circulation in CAR.

A25 Unveiling the relationship between Ceftobiprole and High Molecular Mass (HMM) PBPs

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Low-affinity PBP4 has historically been associated with penicillin-resistance in *Enterococcus faecalis*, though maintaining a potential affinity for the novel cephalosporins. Even if *pbp4* mutations may interfere with antibiotics/PBP4 binding, responsible for its overexpression and aminoacidic substitution, ceftobiprole (BPR) is frequently used as therapeutic option.

Our study aims to explore the interaction between BPR and High-Molecular-Mass (HMM) low-reactive PBPs, focusing on PBP4, in Penicillin-Resistant-Ampicillin-Susceptible/ceftobiprole Not-Susceptible (PRAS/BPR-NS) *E. faecalis* clinical isolates.

Through competition assays, class A and B HMM-PBPs of 7 previously characterized PRAS/BPR-NS *E. faecalis* strains were examined using purified membrane proteins and fluorescent penicillin (Bocillin FL), in treated (1/2- 1- 2- 4-fold BPR-MIC values) and untreated conditions. BPR/HMM PBPs interactions were estimated through the determination of 50% inhibitory concentration (IC_{50}) values for ceftobiprole, using fluorescence intensity trend as indicator.

Because of its low affinity, PBP4 was not significantly acylated among all strains. Also, PBP1a and PBP1b showed a similar insensitivity trend, being inhibited only in 2 and 3 strains, respectively. Conversely, the affinity of the other PBPs to ceftobiprole was variable, with IC_{50} values ranging from 1/2-fold to 4-fold MICs. In two isolates, all PBPs were inhibited already at 1/2-fold the MIC concentration, except for PBP4. Nonetheless, raising BPR concentration, the PBP4 inhibition percentages increased in all strains.

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Our results support the hypothesis that PBP4 is necessary but not sufficient for BPR resistance, changing the paradigm for enterococcal cephalosporin resistance. Our proposition is that a cooperation between class B PBP4 and, at least, one bifunctional class A PBP, could be necessary to synthesize peptidoglycan and promote growth.

A26 Study of the regulatory cascade involved in the megaplasmids conjugation in the model plant symbiotic bacterium Sinorhizobium meliloti by the creation of a GFP-based reporter system

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Sinorhizobium meliloti is a model plant symbiotic bacterium. Its genome is composed by a chromosome, and two megaplasmids, pSymA and pSymB, which carry genes related to the processes of symbiotic nitrogen fixation and growth in the rhizosphere, respectively. The conjugal transfer of megaplasmids in *S. meliloti* is highly regulated and repressed through a system named rct, composed by 7 key genes. However, to date the environmental signals which trigger the rct regulatory cascade are still unknown. Here, we aim to decipher the signals of rct activation. Promoters of rct genes were amplified and cloned upstream to green fluorescent protein (GFP) gene in the pOT2 plasmid in E. coli. We transferred recombinant plasmids to *S. meliloti* Rm1021 strain and tested the induction conditions in presence of a recipient strain of *S. meliloti* Rm1021 lacking the megaplasmid pSymA and root exudate or plant macerate. Levels of fluorescence

of GFP were measured through a cytofluorimeter and results showed that the expression of the different genes belonging to rct system didn't change in response to the tested conditions. However, further studies are necessary to investigate the conditions and the chemical nature of the inducing molecules and the interplay between rtc and the quorum sensing system on the conjugational transfer of megaplasmids.

A27 Desiccation induces apparent death in the pathogenic bacterium *Acinetobacter baumannii*

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Acinetobacter baumannii is a bacterial pathogen endowed with an extraordinary ability to withstand desiccation. This trait, combined with the ability to survive in the absence of nutrients, facilitates the persistence and dissemination of *A. baumannii* in healthcare settings. Although the long-term survival of *A. baumannii* on dry surfaces has a negative impact on infection control measures, the mechanisms at the basis of its desiccation resistance are still elusive.

The adaptive response to long-term desiccation was investigated in two *A. baumannii* strains, namely the type strain ATCC 19606^T and the prototypic epidemic strain ACICU.

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During desiccation, both strains endure structural and functional impairments, which reflect in a dramatic decrease in culturability and virulence in the *Galleria mellonella* larvae model of infection. Single-cell analyses revealed the existence of bacterial sub-populations capable of transitioning to a viable but nonculturable (VBNC) state after desiccation. Resuscitation experiments showed that VBNC cells can repair the incurred damages, fully restoring their culturability and virulence upon rehydration in isotonic buffers. Moreover, transcriptomic analyses substantiated the entrance in a dormancy state for both strains by showing a change of metabolic fluxes to alternative pathways, responsible for bacterial quiescence. Knock-out of genes encoding key enzymes implicated in desiccation-induced alternative pathways resulted in reduced desiccation resistance and resuscitation efficiency. These findings highlight that transition in the VBNC state is the main strategy to survive desiccation and pave the way to the identification of new targets for the development of resuscitation inhibitors.

A28 Characterization of novel *Pseudomonas aeruginosa* genetic determinants associated with pathogenicity in cystic fibrosis lung infections

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Pseudomonas aeruginosa (Pa) causes chronic infections in cystic fibrosis (CF) patients leading to a rapid deterioration of lung function and premature death. *Pa* infections are challenging to eradicate despite the host immune response and sustained antibiotic therapies. Understanding the determinants that lead to *Pa* persistence in the host is extremely important.

Transcriptional analysis of *Pa* communities from sputum samples in CF patients suggests *Pa*

growth in the airways is associated with a specific transcriptional profile. Several genes expressed explicitly in CF lungs are poorly characterized. We hypothesized that among these genes, we might be able to recover new virulence determinants involved in *Pa* persistence.

Due to its homology to a virulence determinant in *Bordetella pertussis*, we selected the PA14_28530 gene for further characterization. *In silico* promoter analysis and *in vivo* genetic experiments based on transcriptional reporter strains allowed us to link the expression of the PA14_28530 to the alternative sigma factor AlgU, a critical regulator of *Pa* virulence.

In addition, we evaluate the effect of PA14_28530 deletion on several virulence-related phenotypes, *i.e*, biofilm formation, antibiotic resistance, and pathogenicity in the *Galleria mellonella* model. Our results suggest that PA14_28530 negatively regulates all the *Pa* virulence traits tested. To understand the molecular mechanisms behind PA14_28530 effects, we performed a genetic screening based on a two-hybrid system, identifying another protein of unknown function (PA14_45850) as a potential PA14_28530 interactor. Further experiments are underway to unravel the role of PA14_28530 and its interactor PA14_28530 in controlling virulence determinants in *Pa*.

A29	**Role of hydrogen sulfide production in *Pseudomonas aeruginosa* antibiotic resistance**

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Recent studies indicate that endogenous production of hydrogen sulfide (H_2S), a well-known inhibitor of terminal oxidases, has a protective effect against antibiotics in several bacteria, including the multidrug resistant pathogen *Pseudomonas aeruginosa*. Hence, enzymes involved in H_2S synthesis could be considered promising targets for the development of new antibiotic adjuvants against *P. aeruginosa*.

The present work aims at investigating the role of H_2S in *i*) antibiotic resistance in the model strain PAO1 and in *P. aeruginosa* cystic fibrosis (CF) isolates; *ii*) the mechanism by which *P. aeruginosa* resists to endogenously produced H_2S.

A set of *P. aeruginosa* PAO1 mutant strains with single and multiple deletions in genes possibly involved in H_2S synthesis/oxidation or coding for terminal oxidases was generated. An optimized protocol based on paper strips soaked with lead acetate allowed determining H_2S levels in PAO1 mutants and in *P. aeruginosa* CF isolates. MIC assays performed on wild type PAO1, selected PAO1 isogenic mutants showing the highest and lowest H_2S-production, and CF isolates allowed studying H_2S involvement in antibiotic resistance. O_2 consumption assays were then used to investigate H_2S sensitivity of *P. aeruginosa* mutants in terminal oxidases.

In this work, we *i*) clarified the role played by distinct *P. aeruginosa* H_2S-synthesizing and -consuming enzymes on H_2S homeostasis, *ii*) showed that production of H_2S in *P. aeruginosa* is not involved in antibiotic resistance, *iii*) demonstrated a protective role of the terminal oxidase CIO in growth conditions that exacerbate H_2S production.

A30 The genetic background and culture conditions only marginally affect the evolutionary trajectories of *Pseudomonas aeruginosa* towards colistin resistance

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BACKGROUND. Colistin represents a last-resort treatment option for *Pseudomonas aeruginosa* multidrug-resistant infections, but colistin resistance is emerging. In *P. aeruginosa*, colistin resistance is always associated with chromosomal mutations that induce the aminoarabinosylation of lipopolysaccharide (LPS). However, several studies have shown that the effect of LPS aminoarabinosylation on colistin resistance varies among *P. aeruginosa* strains and/or experimental settings, and that many other mutations, unrelated to LPS modifications, can influence the extent of colistin resistance.

OBJECTIVES. To verify whether the evolutionary trajectories leading to high-level colistin resistance in *P. aeruginosa* are conserved in different genetic backgrounds and/or under different culture conditions.

METHODS AND RESULTS. *In vitro* evolution experiments in the presence of increasing colistin concentrations were performed for two phylogenetically-distant reference strains, that represent the two major *P. aeruginosa* lineages, in a standard laboratory medium and in media that mimic *P. aeruginosa* growth during infections (human serum and artificial sputum medium). A representative number of colistin resistant clones were subjected to whole genome sequencing to assess whether different strains follow similar evolutionary trajectories regardless of growth conditions or whether some mutations have a strain- and/or growth condition-specific impact on colistin resistance. This analysis revealed that most colistin-resistant mutants share mutations in genes that can be clustered in five functional groups: LPS modification regulators; LPS biosynthesis; polyamine biosynthesis; fatty acid metabolism; outer membrane protein assembly. The generation of deletion/conditional mutants and recombinant strains is in progress to characterize the contribution and mode of action of these pathways during the acquisition of colistin resistance.

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A31 Targeting Tryptophan Synthase to fight *Mycobacterium tuberculosis*

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Tryptophan synthase (TrpAB) is an essential enzyme for bacterial growth, but absent in humans, thus representing a good target for antitubercular drugs. TrpAB is an heterotetramer which catalyses the conversion of indole-glycerol-phosphate (IGP) and L-Serine into L-Tryptophan. In particular, the α-subunits hydrolyze IGP into glyceraldehyde-3-phosphate and indole, while the β-subunits catalyze the reaction of indole with L-Ser to produce L-Trp.

In this context, GSK3778839 (GSK839) compound was identified as a potent antitubercular, active against *Mycobacterium tuberculosis* clinical isolates, displaying no cross-resistance with antitubercular drugs and low Frequency or Resistance. Sequencing of isolated GSK839-resistant mutants suggested an involvement of TrpAB in its mechanism of action. To confirm this hypothesis, the enzyme was produced and characterized. GSK839 was demonstrated to inhibit the β-subunit (IC_{50} 0.89±0.04 μM), without affecting the α-reaction. An in deep characterization revealed that GSK839 is as a mixed uncompetitive inhibitor, with a K_i value of 0.25±0.01 μM, and a residence time of 1.5 min. To further confirm TrpAB as a good target for the development of new drug candidates, different chemical class inhibitors have been assayed against either the α-reaction and the β-reaction. Some compounds proved to be able to inhibit both enzyme activities, but without any particular specificity against one of the two reactions, conceivably affecting the allosteric regulation rather than a specific active site. This study demonstrates the chemical tractability of TrpAB, having the possibility to be inhibited by different chemotypes.

This work has received support from the Innovative Medicines Initiatives 2 Joint Undertaking (grant No 853989).

A32 *In vivo* characterisation of the essential regulator HP1043 in *Helicobacter pylori* and screening of potential inhibitors

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Helicobacter pylori is a highly widespread human pathogen recognised as a class I carcinogen by the World Health Organization. It is the causative agent of several gastrointestinal diseases such as peptic ulcer, gastric adenocarcinoma, and MALT lymphoma. The *H. pylori* genome encodes a small number of transcriptional regulators, including four two-component systems and two orphan response regulators, HP1021 and HP1043. The latter, classified as a member of the OmpR/PhoB subfamily of transcriptional regulators, is essential for cell viability and is involved in the regulation of crucial cellular functions. In this work, we designed a versatile reporter system to characterise HP1043 binding and activity *in vivo*. Given the impossibility of eliminating *hp1043* from the genome, we introduced mutations in the consensus sequence, characterizing the regulator binding site, in order to compare the activity of the reporter system in the presence and absence of HP1043. *In vitro* DNA-binding assays confirmed a modulation of HP1043 interaction upon point mutations of the consensus binding sequence of target promoters. The analysis of reporter gene transcription by qRT-PCR revealed an ambivalent activity of this regulator that shows both activation and repression of the transcription of target genes.

Given the HP1043 essential role for *H. pylori* viability, it represents an ideal target for the development of new antimicrobial strategies against this major human pathogen. In view of this, we screened a library of commercially available compounds for candidates that bound the protein dimerisation site or the DNA binding region. Among them, three molecules inhibited the *in vitro* DNA binding activity of HP1043 in a concentration-dependent manner, supporting the idea of using HP1043 as a potential therapeutic target in *H. pylori* infection.

A33 SigH stress response mediates killing of Mycobacterium tuberculosis by activating nitronaphthofuran prodrugs via induction of Mrx2 expression

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The emergence of drug-resistant Mycobacterium tuberculosis strains highlights the need to discover anti-tuberculosis drugs with novel mechanisms of action. Here we discovered a mycobactericidal strategy based on the prodrug activation of selected chemical derivatives classified as nitronaphthofurans (nNFs) mediated by the coordinated action of the sigH and mrx2 genes. The transcription factor SigH is a key regulator of an extensive transcriptional network that responds to oxidative, nitrosative, and heat stresses in M. tuberculosis. The nNF action induced the SigH stress response which in turn induced the mrx2 overexpression. The nitroreductase Mrx2 was found to activate nNF prodrugs, killing replicating, non-replicating and intracellular forms of M. tuberculosis. Analysis of SigH DNA sequences obtained from spontaneous nNF-resistant M. tuberculosis mutants suggests disruption of SigH binding to the mrx2 promoter site and/or RNA polymerase core, likely promoting the observed loss of transcriptional control over Mrx2. Mutations found in mrx2 lead to structural defects in the thioredoxin fold of the Mrx2 protein, significantly impairing the activity of the Mrx2 enzyme against nNFs. Altogether, our work brings out the SigH/Mrx2 stress response pathway as a promising target for future drug discovery programs.

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A34 Hydrogen sulfide production is dispensable for virulence in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an extremely versatile microorganism equipped with a wide range of virulence factors, making it able to colonize various biotic and abiotic environments outcompeting other microbes. It is recognized as a prototype of Gram-negative multidrug-resistant “superbug” for which new therapeutic strategies are urgently needed.

Hydrogen sulfide (H_2S) is a key endogenous signaling molecule in mammalian (patho)physiology. Genomic investigations revealed that most of the mammalian enzymes involved in H_2S metabolism are encoded in Prokaryotes too. Recently, a role played by H_2S in virulence has been suggested for several bacterial pathogens, including *P. aeruginosa*. Moreover, H_2S is a well-known inhibitor of terminal oxidases, indicating a possible role for this molecule in *P. aeruginosa* competition with H_2S-sensitive bacteria.

Here, we make us of *P. aeruginosa* PAO1 mutant strains that produce higher and lower levels of H_2S relative to wild type PAO1 to investigate the impact of H_2S production on *P. aeruginosa* virulence potential. *In vitro* assays indicated that H_2S production is dispensable to produce key virulence factors, including pyocyanin, proteases, and quorum sensing signal molecules, and to express virulence traits such as motility and biofilm formation. These data are in line with RNA-seq data demonstrating that H_2S has no impact on the transcriptional profile of *P. aeruginosa*

PAO1. Experiments aimed at assessing the possible impact of H_2S production in *P. aeruginosa* virulence *in vivo* (*i.e.* virulence assays in *Galleria mellonella* larvae) and in niche competition with other bacterial pathogens (*e.g. Staphylococcus aureus* and *Acinetobacter baumannii*) are in course.

A35	**The mobilome-enriched genome of the competence-deficient *Streptococcus pneumoniae* BM6001, the original host of ICE Tn5253, is phylogenetically distinct from historical pneumococcal genomes**

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Streptococcus pneumoniae is an important human pathogen causing both mild and severe diseases. In this work, we determined the complete genome sequence of the *S. pneumoniae* type 19F clinical isolate BM6001 which is the original host of the composite ICE Tn5253. BM6001 mobilome (15.54% of the genome) also include the novel IME Tn7089, the novel transposon Tn7090, 3 prophages and 2 satellite prophages, 5 genomic islands (GIs), 72 insertion sequences, 69 RUPs, 153 BOX-elements and 31 SPRITEs. Annotation of the GIs genes revealed the presence of 5 predicted transport systems, which are possibly involved in pneumococcal virulence. Furthermore, ϕBM6001.2 contains virulence determinants encoding a YopX-homolog and the VapE protein, while Tn7090 encodes proteins likely involved in the uptake and binding of Mg2+ cations, in the adhesion to host cells and intracellular survival. All MGEs, except for the GIs, produce excised circular forms and *att*B sites restoration. Since prophage ΦBM6001.3 disrupts the competence *comGC/cglC* gene, we investigated whether prophage mitomycin C induction could restore competence for genetic transformation. Treatment with mitomycin C resulted in a 10-fold increase in the frequency of ΦBM6001.3 excised forms and *comGC/cglC* coding sequence restorations, but did not restore competence for genetic transformation. In addition, phylogenetic analysis showed that BM6001 clusters in a small lineage with other 5 historical strains, but it is distantly related to the lineage due to its unique mobilome. Altogether, our findings suggested that BM6001 has progressively accumulated many MGEs while losing competence for genetic transformation, to become a sort of genomic *cul-de-sac*.

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A36	**Functional characterization of G-quadruplexes in *Mycobacterium tuberculosis***

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Mycobacterium tuberculosis (*Mtb*), the etiologic agent of tuberculosis, is the first cause of death from single infectious disease. In the last decades, the emergence of *Mtb* drug-resistant strains is further pushing research towards the discovery of new therapeutic targets. G-quadruplexes (G4) are non-canonical secondary DNA structures that form in Guanine-rich sequences and are involved in several fundamental biological processes. We have previously identified putative

G4s in *Mtb* genome and showed that G4-ligand molecules, such as BRACO-19, are able to inhibit bacterial growth. Our current goal is to understand the biological function of G4s in *Mtb*. We, therefore, performed RNA-seq upon treatment with the G4-ligand BRACO-19. We found a correlation between the presence of putative G4s in the coding regions of the differentially expressed genes (DEGs) and the level of expression, suggesting a relationship between the presence of G4s and gene expression. We tested the hypothesis that the presence of G4s in the coding sequence confers stability to the RNA filament. Our results show that only induced genes having G4s in the coding sequence increase their half-life upon BRACO-19 treatment. We will further study the role of the G4s of interest *in vitro* and *in vivo* by mutagenesis experiments.

A37 Isolation and Identification of Lactic Acid Bacteria from Natural Whey Starter Culture of Cow Milk

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The growing market of probiotic food products stimulates scientific research towards the continuous characterization for new probiotic strains. Natural whey starter (NWS) cultures are among the best sources, since they are characterized by high microbial diversity. We studied the microbial community of NWS culture of cow's milk for the production of caciocavallo. Lactic acid bacterial identification from NWS at species and strain level was based on culture-dependent strategy, including enumeration and isolation of lactic acid bacteria on selective media, sequencing of the 16S rDNA and species/subspecies specific Polymerase Chain Reaction (PCR). Results obtained showed the occurrence of different LAB including *Lactococcus lactis* subsp. *lactis*, S*treptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum* and *Lactobacillus helveticus* strains. By RAPD analysis we have also evaluated the genetic diversity of all isolates. Moreover, microbial community analysis by Next Generation Sequencing (NGS) is currently under study. Characterization of the microbiota of natural whey starters aims to collect new starter bacteria to use for tracing microbial community during the production of artisanal cheeses, in order to preserve their quality and authenticity, and to select new Lactic Acid Bacteria (LAB) strains for the production of functional foods.

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A38 Insight the glycopeptidolipids gene cluster in *Mycobacteria*: isolation of *ΔMSMEG_0394* mutant strain

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In recent years the worldwide incidence of non-tuberculous mycobacteria (NTM) infections has dramatically increased. Among known NTM, *Mycobacterium abscessus* is the most difficult to manage. Glycopeptidolipids (GPLs) are the most representative class of glycolipids present in the outer layer of several species of NTM. These molecules play an important role in both mycobacteria physiology and pathogenicity. Here we study the GPLs gene cluster using *Mycobacterium smegmatis* as model organism. All genes necessary for GPLs biosynthesis in NTM are clustered in a single highly conserved region of 65 kb. Using *in silico* analysis, we identified within this region,

the *MSMEG_0394* gene, whose predicted protein product share 75% amino acid identity with *M. abscessus* MAB_410c gene product. Both putative proteins are annotated as “hypothetical” with unknown function. By RT-PCR transcriptional studies we demonstrated that *MSMEG_0394* is the second gene of an operon that contains *MSMEG_0393/94/95* genes. In order to verify if *MSMEG_0394* gene product is involved in GPL biosynthesis, we have isolated a mutant strain of *M. smegmatis* carrying a null mutation in the *MSMEG_0394* gene. *M. smegmatis* Δ0394 was engineered using the p2NIL/pGOAL19-based flexible cassette method. Positive clones, selected to carry a deletion and no residual plasmid DNA in flanking regions, have occurred in 5% of the total recombinant clones. Phenotypic analysis of the mutant strain is under study. The study of mycobacterial mutants defective for GPLs is crucial to identify new antimicrobial molecules useful for the development of therapeutic strategies based on treatment with innovative drug formulations.

A39 Isolation and characterization of different bacteriophages of *Pseudomonas aeruginosa* for therapeutic use

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Pseudomonas aeruginosa is a critical priority pathogen, due to its ability to easily select drug-resistant clones. New anti *P. aeruginosa* strategies are thus mandatory.

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The use of bacteriophages for therapeutic purposes (phage therapy) represents one of the most promising solutions for the treatment of difficult-to-treat infections. To avoid the onset of phage-resistance and to expand the host-spectrum, a phage cocktail is preferred to mono-phage preparations. The aim of this study was to isolate and genotypically characterize new bacteriophages targeting *P. aeruginosa*, and evaluate their lytic activity against clinical isolates.

Forty phages were isolated from twenty-eight environmental samples following enrichment techniques, using three multi-drug resistant *P. aeruginosa* clinical isolates from different European countries as hosts. Phage genomes were sequenced (Illumina) and annotated. Lytic activity of isolated phages was assayed by spot assay against a panel of thirty-three *P. aeruginosa* clinical strains to evaluate their host-spectrum.

Bioinformatic analysis of sequenced genomes revealed seventeen different phages, of which twelve were virulent, while the remaining five resulted temperate ones. No virulence or antibiotic-resistance genes were detected in the genomes of virulent phages, except in two cases. Genes coding for a toxin and a hypothetical efflux pump were detected in two different lytic phages, respectively. All clinical isolates (except one), no matter their geographic origin were susceptible to phages.

In conclusion, the virulent nature, the lack of virulence and/or antibiotic resistance genes and the broad host-range against several *P. aeruginosa* strains in ten isolated phages suggest the possibility of combining them in therapeutic phage cocktails.

A40 Characterization of Mutants Involved in Cell Wall Synthesis and Remodeling in *Acinetobacter baumannii*

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The emergence and spread of multi- or pan-resistant nosocomial pathogens characterized by a tremendous ability of intrahospital dissemination is of extreme critical relevance. Among them, *Acinetobacter baumannii* is a priority pathogen that requires urgent strategies of intervention. The cell envelope is an essential structure that maintains cellular integrity and provides protection against external stresses. Characterizing pathways involved in the regulation and remodeling of peptidoglycan (PG) and its integrated function with the outer membrane can lead to the identification of new targets with lower evolutionary pressure and thus less likely to promote the development of resistance. In this work, we phenotypically characterize *A. baumannii* AB5075 knock-out mutants for non-essential proteins involved in PG synthesis regulation and remodeling under physiological and stress conditions to identify proteins that can affect fitness, virulence, and antibiotic resistance. Mutants inactivated for Penicillin-binding proteins, L,D-transpeptidases and regulators of PG synthesis displayed clear morphological defects, despite that none of them showed a significant difference in growth and viability in comparison to the WT. Antibiotic susceptibility assays confirmed for *A. baumannii* AB5075 a general profile of resistance to several antibiotics targeting the cell envelope, except polymyxins, and possible heteroresistance with carbapenems. These findings highlight the importance of studying how proteins involved in PG synthesis regulation and remodeling are orchestrated to allow proper growth and division. Besides its interest as a clinically relevant and antibiotic resistant pathogen, *A. baumannii* provides also a unique model among Gram-negatives to study the relationships between the different components of the cell envelope.

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A41 Adaptive Potential Of Marine Symbioses To Climate Change: Insights From Non Model Organism Manila Clam

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Extreme climatic events like Marine Heatwaves (MHWs) are becoming more intense and frequent and are severely threatening ecosystems' health. Specifically, marine ecosystems are facing an increase in the prevalence of pathogenic microbes, causing significant shifts in the ecology and evolution of animal-microbiome symbiosis in marine environments. However, how extreme climate events shape the evolution and the adaptation of marine hosts and their microbiomes is still poorly understood. Here, we characterized the effect of heat waves on the microbiome of Manila clam Ruditapes philippinarum, one of the most widely farmed clam species worldwide, and investigated the ultimate effect on host health.

We found that thermal stress significantly affect different animal fitness traits (e.g. reduced energy reserves, impaired reproduction, altered behaviour and filtration rate), as well as clam microbiome by decreasing the overall bacterial richness and providing a suitable environment for pathogens to thrive. Specifically, we found that a distinct subset of microorganisms competes to establish a new

microbiome in challenged clams. The relative abundance of beneficial symbionts decreased by 10-fold in challenged clams, while that of Vibrio spp., frequent cause of clam infections, increased by 18-fold.

Next, by generating gnotobiotic clams, we investigated the effect of microbiomes adapted to extreme climate events in protecting their host from fitness costs caused by heat stress. We will report the results about the effect of MHWs to clam-microbiome symbiosis together with the results on the adaptation potential of microbiomes to heat stress and their ultimate effect in improving host resilience to climate change.

A42 First insights into the epigenetic mechanisms regulating lipid biosynthesis in *Rhodococcus* bacterial strains

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DNA methylation is an epigenetic regulatory mechanism generally present in both eukaryotic and prokaryotic organisms and plays an important role in numerous cellular systems and processes. The main studies on DNA methylation in bacteria have focused on pathogenic bacteria and/or Gram-negative model strains. Only a few works have investigated the role of DNA methylation in Gram-positive bacteria and even fewer have analysed the regulatory role in bacterial gene expression with biotechnological relevance/applications. In this process, the DNA methyltransferases (MTases) catalyze post-replicative modification on DNA by transferring a methyl group to adenosine or cytidine bases in DNA. Here, we provide first insights into the role of DNA methylation in the regulation of genes involved in triacylglycerol (TAGs) in the oleaginous strain *Rhodococcus (R.) opacus* PD630. Through PacBio single molecule real-time (SMRT) sequencing, we conducted methylomic analysis of the PD630 WT under nitrogen limiting condition that is associated with TAGs production and accumulation, and we compared the methylation profiles with the non-limiting growth conditions. This analysis allowed to identify different methylation motifs. Furthermore, several promoters were differentially methylated under N-limiting condition suggesting a possible role of DNA methylation in TAGs biosynthesis in *R.opacus* PD630. We also identified two genes encoding methyltransferases (MTase) in the PD630 genome through a bioinformatic approach and we constructed the deletion mutant of the MTase located on the chromosome. The analyses of this mutant considering the lipid production yield and the growth performance o n different growth media/carbon sources (inclusing aromatic hydrocarbons) are presently under investigation.

A43 The TAM complex: a novel key player of the outer membrane homeostasis of Bacteroidetes

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In Proteobacteria, the outer membrane (OM) protein TamA forms with the inner membrane (IM)-anchored periplasmic protein TamB the Translocation and Assembly Module (**TAM**) complex, which transports autotransporters and virulence factors. Recently, TAM was shown to be involved in the anterograde (IM to OM) phospholipid transport, which indicates that TAM is also involved in lipid trafficking across the two membranes.

In Bacteroidetes, TamB co-occurs with **TamL**, a TamA-like lipoprotein structurally similar to TamA but with an N-terminal lipid moiety, which likely anchors the N-terminus to the OM, though the biological significance of this is unknown. Interestingly, in some Bacteroidetes, such as *Flavobacterium johnsoniae* (*Fj)* and *Capnocytophaga canimorsus* (*Cc*), the former an environmental bacterium, the latter a dog and cat oral commensal and human pathogen, the TamL and TamB encoding genes are both essential, unlike their homologues in Proteobacteria. Furthermore, neither *Fj* nor *Cc* possess autotransporters encoding genes. These observations suggest that TamL and TamB may have novel functions restricted to Bacteroidetes. To this aim, we performed TamB/L pull-down assays, coupled with mass spectrometry (MS) analyses, to identify those proteins TamL and TamB interact with. In parallel, we depleted TamB/L to characterize the effect of their depletion on cell viability and OM protein and lipid composition.

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Here we show that depletion of TamL or TamB has a detrimental effect on cell viability and leads to morphological abnormalities, which may indicate an effect on OM integrity. Additionally, we discuss the impact TamL depletion has on OM protein composition, and our preliminary pull-down results.

A44 Systematic predictions of *Fusobacterium nucleatum* physiology by integrating transcriptomics and growth data

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Bacteria living inside the tumoral micro-environment play a crucial role in the development of cancer and its progression. Enrichment of *Fusobacterium nucleatum* in colorectal cancer (CRC) tissue has been acknowledged as a major driver of its proliferation and mortality. Representatives of the *F. nucleatum* species exhibit a remarkable variability, being linked to a growing list of disease, however it is still unclear how this can be related to increased virulence or to association with cancer.

In this work we aimed at characterizing the basal physiology of the bacterium in a rich environment, to produce a predictive computational platform (FNREC) to simulate the phenotypical features of *F. nucleatum* in different conditions. We used gene expression data obtained from *in vitro* models

to simulate physiological features of this bacterium, allowing us to identify essential genes and metabolites of pathological relevance. In conclusion, FNREC can be used as a computational platform to gain insights into the biology of this bacterium, increasing our understanding of genotype-phenotype relationships to design targeted experiments.

A45	**Defeat *Staphylococcus aureus* by targeting multiple iron acquisition systems**

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Staphylococcus aureus is a bacterial pathogen causing a broad spectrum of human diseases. *S. aureus* has reached an alarming high level of antibiotic resistance, leaving few viable therapeutic options to clinicians, and urgently calling for novel anti-*Staphylococcus* treatments.
Given the crucial role of iron in bacterial physiology and pathogenicity, iron uptake and metabolism have been evaluated as possible drug targets.
During infection, *S. aureus* acquires iron from heme, the preferred iron source, *via* the IsdB and IsdH hemophores, and other proteins (also belonging to the Iron-regulated surface determinant Isd-system) able to deliver iron to the cytoplasm. Moreover, *S. aureus* can also acquire iron by producing two siderophores, namely staphyloferrin A (SA) and staphyloferrin B (SB).
As part of the ERASE multidisciplinary project (PRIN2020AE3LTA), the aim of this study is to investigate two mechanisms of iron acquisition by *S. aureus*: heme scavenging by IsdB/IsdH and iron uptake by SB, setting up the conditions to assay possible inhibitors of these processes, hence of *S. aureus* growth.
We have determined suitable growth conditions to investigate the effect of iron starvation in S. *aureus*, represented by the iron-deplete medium cTMS (Chelex-treated Tris Minimal Succinate), in which high levels of siderophore were produced by *S. aureus*. Moreover, the addition of Hemoglobin or $FeCl_3$ in cTMS promoted bacterial growth, thus confirming that iron is perceived as a limiting nutrient in this medium.
Additionally, an *isdB in frame* deletion mutant has been successfully generated and its contribution to the growth of *S. aureus* is currently under investigation.

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A46	**Reverse Vaccinology: Unveiling Antigen Candidates for the development of a vaccine against the *Burkholderia cepacia* Complex**

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Burkholderia cepacia complex (Bcc) bacteria can colonize immunocompromised, hospitalized and people with Cystic Fibrosis. Since they are naturally resistant to antibiotics, empirical treatments are often unsuccessful. Considering the limitations in developing new antibiotics, vaccination could be used to prevent diffusion of resistant strains and protect fragile patients.
As no vaccine for Bcc bacteria is available yet, in this work we exploited the reverse vaccinology approach to search for antigen candidates.
The annotated genomes of 16 Bcc bacteria were compared and proteins were sorted according to conservation, predicted localization, immunogenicity, and function, identifying 24 proteins. Three

candidates -BCAL1524, BCAM0949, and BCAS0335- were selected for further characterization; after producing deletion mutants, their localization in the Outer Membrane Vesicles was assessed and different aspects of virulence were investigated.

While none of the proteins studied affected bacterial growth, BCAL1524, a collagen-like protein, promoted bacterial autoaggregation and virulence in *Galleria mellonella*. BCAM0949, an extracellular lipase, mediated piperacillin resistance, biofilm formation in LB and Artificial Sputum Medium, rhamnolipid production, and swimming motility; its lipolytic activity was also assessed. BCAS0335, a trimeric autotransporter adhesin, promoted minocycline resistance, biofilm organization in LB, and virulence in *G. mellonella*. Interestingly, BCAM0949 showed phenotypic similarities with *P. aeruginosa* EstA, a key virulence factor.

By providing the deleted genes *in trans*, each phenotype was complemented, thus validating the role of each protein in virulence. Given the promising results achieved, additional experiments will be fundamental to evaluate the immunogenicity of these antigen candidates in a mouse model.

A47 Deciphering the role of multidrug efflux pumps in the virulence of enteroaggregative *Escherichia coli*

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Efflux pumps (EPs) constitute an important group of membrane proteins that characterize all living organisms, from bacteria to humans. Acting as intermembrane transporters, they perform a crucial role in the extrusion of different types of compounds from the cell, and their overexpression constitutes one of the main mechanisms employed by bacteria to implement multidrug resistance (MDR). Although MDR EPs are mainly involved in antibiotic excretion mechanisms, many other functions have been attributed to them including communication with the external environment and host cell, stress response, and biofilm formation. Despite their relevance in many pathogens, up to now no data are available on the potential role of MDR EPs during the pathogenicity process of enteroaggregative *Escherichia coli* (EAEC). EAEC is a group of enteropathogenic *E. coli* that are known for their ability to form thick biofilms on the intestinal mucosa and represents the leading cause of acute and persistent diarrhoea worldwide. Using the clinical strain EAEC 17-2 as a model, we first identified in silico the genetic loci encoding MDR EPs. Then, by site-specific mutagenesis, we isolated mutants in genes encoding MDR EPs known to be involved in biofilm biogenesis in commensal *E. coli*. Preliminary results indicated that AcrAB, the major MDR EP, is implicated in biofilm formation and maturation as the loss of the AcrB transporter leads to a significant reduction of extracellular DNA in the biofilm matrix.

A48 Synthesis and applications of a far-red emitting fluorophore set in bacterial live-cell imaging, membrane staining and nanoscopy

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Advances in fluorescence microscopy come accompanied by increasing demand for novel fluorophores that enable tagging specific cellular components. Fluorescent probes emitting in the far-red (FR) are extremely versatile in fluorescence microscopy since they reduce possible interference caused by sample autofluorescence and increase the flexibility in multicolor imaging experiments. The lack of FR-emitting fluorophores for bacterial labeling limits the possibility to investigate cellular structures via multicolor imaging.

In this study, an extended set of FR-emitting dyes with different net charges (from -4 to +2) and various functional groups based on rhodamine and oxazine scaffolds has been employed in confocal laser scanning microscopy (CLSM) imaging of *Bacillus subtilis* and *Escherichia coli* cells. Toxicity tests demonstrated that these dyes do not interfere with the growth kinetics of both species, opening the possibility to use them in live-cell imaging. Moreover, all the dyes can discriminate viable from dead bacterial cells. Among the newly synthesized fluorophores, the oxazine derivative KK 1905-NHS was particularly efficient in membrane staining and was effectively employed to monitor membrane biogenesis using a two-step labeling protocol on living cells. Thanks to its high photostability, KK 1905-NHS was successfully used in stimulated emission depletion (STED) microscopy, a super-resolution technique capable to increase the optical resolution of CLSM up to one order of magnitude but requiring photostable fluorophore.

Overall, the new fluorophores presented in this study expand the microscopy toolbox available to bacteriologists, providing an asset for the investigation of fundamental biological processes in bacteria.

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A49 Characterization of Multidrug Resistance *Klebsiella pneumoniae* strains from *Lesser kestrels*

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Antimicrobial Resistance (AR) and the progressive isolation of multidrug resistant bacteria (MDR) from clinical and environmental samples, have been recognised as a global public health problem. The upsurge of AR is mainly due to both horizontal transfer, mostly mediated by conjugative plasmids (CPs), of antimicrobial resistance genes (ARGs) and the spread of MDR clonal strains. Within this framework wild animals have recently been found to play a non-secondary role. In this study we report preliminary data on detection and characterisation of MDR *Klebsiella pneumoniae*

strains (KP) isolated in Apulia in 2021 and 2022 from *Lesser kestrels*. Clonal relatedness assessed by pulsed-field gel electrophoresis (PFGE), established the presence of two distinct and clonally unrelated groups of strains: the 2021 and 2022.
The 2021 strains harboured a conjugative (frequency of $1x10^{-6}$ donors/transconjugants) IncFIIk/IncFI-B plasmid (223.558 bp). The conjugative plasmid included two regions of which one carried genes encoding resistance to heavy metals (As, Ag and Cu) and an iron uptake system; the second ARGs (*aac(3)-IIa, aac(6')Ib-cr, strAB*), (*bla*$_{CTX-M-15}$, *bla*$_{TEM1B}$, *bla*$_{OXA-1}$), (*qnrB1*), (*dfrA14,sul2*) and (*tetA*), encoding resistance to aminoglycosides, beta-lactams, quinolone, trimethoprim-sulfamethoxazole and tetracycline, respectively. Insertion sequence elements of different classes were scattered along the two regions. Noteworthy was the presence of eight IS26 clustered within ARGs.
This preliminary study has highlighted for the first time the potential contribution of *L. kestrels* in the spread of AR. Besides, it poses open questions such as the acquisition of a MDR-CP mainly detected in KP isolated from clinical cases.

A50 Cut&Tag: a novel protocol for profiling transcription dynamics in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis in humans, remains a resilient pathogen worldwide, displaying remarkable adaptability in diverse host environments. Consequently, there is a pressing need to elucidate the mechanisms of gene regulation and DNA-protein interactions to gain a detailed understanding of bacterial physiology.

In this study, we adapt a protocol called Cleavage under targets and tagmentation (CUT&TAG) to develop an innovative approach for chromatin mapping in Mtb.
We performed experiments using an anti-*E.coli* RNA polymerase beta subunit antibody and integrated CUT&TAG data with RNA-seq analysis. We detected peaks across different regions of the genome that are indicative of the interaction of the transcription machinery with DNA; we observed a pronounced enrichment in regions upstream or overlapping the gene start sites. Corroborating our findings, the RNA-seq data demonstrated a significant correlation between the occupancy of the transcriptional complex and the transcriptome.

CUT&TAG is an innovative technique that offers several advantages, compared to ChIP-seq. First, it allows testing the cells with mild cell fixation, thus maintaining more physiological test conditions; second, it requires remarkably lower starting material and eliminates the need for sonication and prolonged crosslinking. Third, it enables a faster experimental timeline. Fourth, it allows for the simultaneous use of multiple antibodies, combining immunoprecipitation with library preparation. Fifth, it requires lower sequencing depths while still enabling the identification of chromatin-interacting proteins.
The applicability of CUT&Tag to bacteria shown in this work makes it the method of choice for genome-wide detection of nucleic acid conformations and binding factors.

A51 Unraveling the rhizobial epigenome and its influence on plant symbiotic interaction

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Although epigenetic phenomena are widely studied in eukaryotes, the role of epigenetic DNA methylation is not fully understood in prokaryotes, where two main families of methyltransferases (MTase) have been identified: the so-called orphan methyltransferases and those that are part of restriction modification (R-M) systems. DNA methylation has been associated to cell cycle control and identification of parental DNA strand.

Within Alphaproteobacteria, rhizobia constitute a key group of plant symbiotic bacteria, carrying out symbiotic nitrogen fixation with host legumes. They show very high strain-level genomic and transcriptomic variability which result in extreme variation in symbiotic performances. May this variability reside on DNA-methylation, either related to R-M systems or promoter activation? Insights into molecular mechanisms and genomic background favoring the exchange of large genetic elements will make possible to develop innovative tools to sustain environmentally friendly agricultural practices.

The aim of this work is to explore the DNA methylation patterns in a population of 23 *Sinorhizobium meliloti* strains. We developed a computational pipeline to analyze PacBio SMRT-derived methylation pattern, parsing the methylated positions within the genome and detecting methylated regions. While each strain shares some DNA methylation pattern, different methylated motifs vary along the genomes, suggesting that they may act as barrier to gene transfer and have a role on gene expression regulation. Furthermore, the occurrence of methylated genes is different when considering regulatory regions among replicons.

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Future work will deepen the statistical and experimental validation of the possible link between methylated motifs and genome-wide horizontal gene transfer events and/or gene co-expression.

A52 Insights into the evolution of multipartite genomes in *Proteobacteria*

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Multipartite genomes, consisting of more than one replicon, have been found in approximately 10% of bacteria, many of which belong to the phylum *Proteobacteria*. Many aspects of their origin, evolution, and the possible advantages related to this type of genome structure remain to be elucidated.

Through a combination of phylogenetic analysis and pangenome-level functional enrichment analyses, we performed a systematic analysis of the presence and distribution of multipartite genomes in different class of *Proteobacteria*, to clarify some aspects of their origin and the possible advantages associated with them.

Our data suggest that the emergence of secondary replicons in *Proteobacteria* is rare and that they derived from plasmids. Despite their multiple origins, we highlighted the presence of evolutionary trends such as the inverse proportionality of the genome to chromosome size ratio, which appears to be a general feature of bacteria with multipartite genomes irrespective of taxonomic group. Secondly, we highlighted some functional trends. The core gene set of the secondary replicons is extremely small, probably limited to essential genes or genes that favour their maintenance in the genome. This hypothesis agrees with the idea that the primary advantage of secondary replicons could be to facilitate gene acquisition through horizontal gene transfer, resulting in replicons enriched in genes associated with adaptation to different ecological niches. Indeed, secondary replicons are enriched both in genes that could promote adaptation to harsh environments, and in functional categories related to the exploitation of environmental resources, which can complement chromosomal functions.

A53 WGS based evaluation of molecular mechanisms potentially involved in reduced susceptibility to cefiderocol in ceftazidime/avibactam resistant *Klebsiella pneumoniae* clinical isolates

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Introduction

Ceftazidime-avibactam (CZA) has been introduced in 2018 to treat serine-beta-lactamase Gram-negative bacteria. CZA resistance in KPC-producing *K. pneumoniae* has emerged.

Cefiderocol (FDC) is a novel siderophore cephalosporin which exploits bacterial siderophores. Since ceftazidime and FDC share structural similarities, previously data suggested that several CZA-resistant KPC-3 variants may have an impact on susceptibility to FDC leading to cross-resistance (doi: 10.1007/s10096-021-04397).

Materials and Methods

A collection of 46 *K. pneumoniae*, was subjected to Illumina and Oxford Nanopore Technologies genome sequencing. Twelve KPC-inhibitor resistant encoding genes were cloned in pTOPO-NeoR vector in DH5-alpha *Escherichia coli* cells. MICs were measured by the FDC COMAsp (Liophilchem).

Results

A collection of 46 *K. pneumoniae*, belonging to 6 major Sequence Type (ST), including ST512, ST101, ST307, ST147 encoding 12 different inhibitor resistant KPC-3 variants (8 of which never described before), were studied at genome level and tested for FDC resistance.

Two strains co-harboring KPC and the VIM metallo-beta-lactamase exhibited the highest MICs for both CZA and FDC. In one of these strains a nonsense mutation in the *cirA* siderophore gene was observed and conferred a MIC>32 mg/L. Reduced susceptibility to FDC was correlated to KPC-68 and KPC-70 variants and confirmed in isogenic *E. coli*.

Discussion and Conclusions

Specific KPC variants in the omega-loop of the protein, conferring high CZA resistance levels may also impact on susceptibility to FDC. However, other factors identified in the genomes seem to contribute (known siderophores, predicted iron transporter) or not to contribute (outer membrane protein mutation), to reduced susceptibility to FDC.

A54 Parrotfish microbiome and its role in the coral reef ecosystem

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Coral reef islands are a unique landforms composed by sediments derived from the surrounding coral reefs, which are particularly sensitive to climate change. Recently, parrotfishes have been identified as the main actor in the formation of such islands. Particularly, excavator parrotfish species contribute for >80% of the new coral sediment production, which constitutes the substrate for reef islands formation. To date, how parrotfish microbial symbionts reacts to coral ingestions and their role in sediment formation remains elusive. To this end, we collected both oral and rectal microbiome samples from 8 different species of parrotfish, as well as corals and seawater samples at the Maldives islands (Magoodhoo reef). Preliminary analyses, based on 16S rRNA amplicon sequ encing, show that the gut ecosystem is richer than the oral one in term of bacterial diversity and that both are poorly influenced by coral microbiome. Moreover, the gut ecosystem is characterized by a strong phylogenetic diversity between the excavator and scraper species, suggesting that the different feeding behaviour, as well as the different intake of coral in their diet shape the composition of the host microbiome.

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A55 A possible involvement of MmpL4 in a new mechanism of action/resistance for the antitubercular drug TBAJ-587 and its metabolites

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TBAJ-587 is a preclinical drug (TB Alliance), developed as a safer diarylquinoline (DARQ) with potential for superior efficacy to bedaquiline (BDQ), the first DARQ in the new regimen for the treatment of drug resistant tuberculosis (TB). Two mechanisms of BDQ resistance are caused by mutations in either *atpE* gene, encoding BDQ target, or in *Rv0678*, encoding the repressor of the MmpS5-MmpL5 efflux pump. TBAJ-587 forms three active metabolites *in vivo* (M2, M12, M3). Within the collaboration with ERA4TB consortium, the mechanism of action (MoA) and resistance of M3 and the other metabolites were studied to confirm that AtpE was their cellular target, as observed for TBAJ-587. The activity of TBAJ-587 and its metabolites was tested against a panel of *Mycobacterium tuberculosis atpE* and *Rv0678* BDQ mutants. The mutants were resistant to M3, M2 and M12 with a range of 8X-30X shift compared to parental strains, confirming that TBAJ-587 and its metabolites share the same target and resistance.
To find a new M3 MoA unrelated to *Rv0678*, spontaneous M3-resistant mutants were isolated from

Mtb Rv0678 mutant (IC1O) and characterized by WGS. Two of M3-resistant mutants showed a deletion at position 1921 (Δ1921) in *mmpL4* gene coding for an essential siderophore transporter during infection. *mmpL4* mutants resulted resistant to M3, M2 and M12, but not to BDQ and TBAJ-587. We are characterizing MmpL4, as a new possible MoA of M3 by microbiological, genetic, and biochemical approaches.

This work has received support from the Innovative Medicines Initiatives 2 Joint Undertaking (grant No 853989).

A56 Characterization of envelope stress responses in Bacteroidetes

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To deal with sudden environmental changes, the bacterial envelope is a dynamic structure that promptly adapts to new conditions. This is achieved thanks to dedicated envelope stress responses (ESR) which have been mainly studied in the model organism *Escherichia coli* but poorly characterized in Bacteroidetes. ESR can be grouped into two main categories: two-component signal transduction systems and the alternative sigma factors associated to the RNA polymerase. An *in silico* search for homologs of the components of known ESR systems revealed that only several RpoE homologs are conserved in the dog oral commensal and human pathogen *Capnocytophaga canimorsus*. This suggests that these systems are either absent or highly divergent.

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Our work aims at (i) characterizing the role of *C. canimorsus* RpoE homologs and (ii) identifying novel ESR systems. To investigate the function of the RpoE homologs, we (i) generated mutants and monitored their growth and sensitivity to several envelope stresses and (ii) we are currently identifying the regulons of the sigma factors by ChIP-seq. In addition, to identify unknown ESR systems, we are performing a transposon sequencing (Tn_seq) analysis in sub-lethal envelope stress conditions.

So far, we found that several *rpoE* mutants have a growth defect in specific envelope stress conditions, suggesting a potential membrane sensitization in these mutants. In addition, the overexpression of several *rpoE* appears to be toxic under different growth conditions. Finally, we identified the putative regulons of an essential *C. canimorsus rpoE* homolog and of two *rpoE* predicted to be involved in OM stress response.

A57 Evolution of quorum sensing regulation through the acquisition of additional feedback loops

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Bacterial quorum sensing (QS) is a cell-to-cell communication system in which specific signals are activated to coordinate, among others, pathogenic behaviours and help bacteria collectively respond to perturbations. QS in Gram-negative bacteria is typically regulated by an N-acyl-homoserine lactone (AHL) molecules-mediated system, homologous of *Vibrio fischeri*'s LuxI-R. In

many cases, bacteria possess more than one QS system, based on different types of molecules, that interact through a complex regulatory network. Presumably, these configurations have emerged over time from simpler ones through the acquisition of novel players (e.g., transcription factors) that have been successfully integrated into the native regulatory systems. However, the advantages provided by these alternative/additional configurations on QS-related phenotypes is poorly predictable only based on their underlying network structure. Here we studied to which extent horizontal gene transfer (HGT) has contributed to the extant distribution of LuxIR-like quorum sensing modules in prokaryotic genomes. Using machine learning and genomic composition analysis we classified LuxI- and LuxR-like sequences of 32,482 prokaryotes into native and non-native (i.e., likely acquired through HGT) and integrated this information on the corresponding phylogenetic tree. Our classification reveals a dynamic gene gain/loss distribution of LuxIR-like systems across >100 species of different phyla. Next, we focused on one specific case, i.e., the well-studied *cci* genomic island previously characterized in the *Burkholderia* genus and known to harbour an “extra” QS regulation module. We investigated the effects of this additional regulation over the native QS system by generating mutants harbouring a reduced (core) regulatory circuit and compared their QS response with the wild-type circuit (complete). Experimental results indicate that one of the effects of an additional QS regulation module resides in its capability to buffer the variability of final cell densities in growing populations of *Burkholderia* cells, thus probably reducing cell-to-cell variation of growth phenotypes. Finally, we implemented a mathematical model that reproduced the experimental observations and that allowed the investigation of other possible consequences of this horizontally transferred QS module. Not only do we propose a scenario in which the additional feedback loops are acquired horizontally, but we also speculate that the original CepIR system might have been transferred multiple times across several bacterial families, thus expanding our understanding of the effects of acquired DNA on existing molecular circuitries. In conclusion, our results illuminate on the possible, non-trivial, phenotypes that may arise because of HGT events.

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A58 Elucidating the role of transition metals availability on microbial metabolism: an *E. coli* K-12 DH5α model study

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Transition metals play an essential role in the metabolism of all living organisms, since most key metabolic enzymes are metal-containing oxidoreductases. In fact, transition metals are included in the structures of cofactors and catalytic domains of most enzymes directly involved in biogeochemical cycles and in all the energetic metabolic pathways, such as aerobic and anaerobic respirations, photosynthesis, carbon and nitrogen fixation, and so on. Although the significant role

of transition metals in microbial metabolism now seems well established, the effects of changes in their availability have not yet been fully demonstrated.

Escherichia coli is able to grow both in aerobic and anaerobic conditions, using oxygen and nitrate as the terminal electron acceptor in the respiratory cascade, respectively. The key enzymes involved in the *E. coli* respiratory pathways are metal-containing oxidoreductases, i.e. cytochrome bo(3) ubiquinol oxidase [copper-iron], cytochrome bd-I and bd-II ubiquinol oxidases [iron], nitrate and periplasmic nitrate reductases [molybdenum].

Our purpose was to investigate the role of transition metals in *E. coli* respiratory pathways, evaluating the growth performances of *E. coli* K-12 DH5α strain in M9 medium in absence and in presence of transition metals, both in aerobic and anaerobic conditions. Our data demonstrate that transition metals, as fundamental components of the key respiratory oxidoreductases, can improve the *E. coli* growth performances and suggest that the differences in their availability can drive into metabolic switches independently from the metabolic substrates amount.

A59 Identification of a new non-canonical structure in the genome of *Mycobacterium tuberculosis*

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Tuberculosis (TB) is caused by the bacillus *Mycobacterium tuberculosis* and is one of the leading causes of death worldwide, as reported in the “Global tuberculosis report 2022”.

The lack of an effective vaccine and the ever-increasing resistance to antibiotics make the search for new anti-TB drugs a significant challenge for biomedical research.

G-rich regions of the genome of *M. tuberculosis* were investigated in order to find a sequence pattern potentially capable of folding into a nucleic acid secondary structure. The aim was to study the epigenetical regulation of cellular functions, as a potential pharmacological target.

We identified CORE-1, a highly repeated sequence within the genome of *M. tuberculosis* but very rarely repeated in the human genome, that is capable of folding in vitro into a G-hairpin.

The G-hairpin is a secondary structure that has recently attracted interest. It consists in a filament folded back on itself forming a loop and stabilized by both Watson-Crick and Hoogsteen bonds.

To investigate the physiological role of CORE-1 G-hairpin, we performed a pull-down assay with mycobacterium proteins that allowed us to identify a protein (Rv1488) interactor of CORE-1, potentially involved in the physiological activity of the structure.

Since Rv1488 is an uncharacterized protein, we are now carrying out some functional assays on both CORE-1 and the protein, with the long term aim of exploiting them as candidate therapeutic targets.

A60 Role of the 2,4 dienoyl-CoA reductase FadH in fatty acid degradation

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Fatty acids (FAs) are an important source of energy and carbon. Saturated FAs are degraded by the conserved enzymes of the β-oxidation cycle. Degradation of unsaturated FAs requires additional auxiliary enzymes. Specifically, unsaturation at an even position requires an additional 2,4 dienoyl-CoA reductase step. Two distinct protein families carry this activity: FadH-like proteins containing an [4Fe-4S] cluster in bacteria, and DECR proteins in eukaryotes.
This study focused on the importance of FadH and its [4Fe-4S] cluster in bacterial physiology. First, we showed that the [Fe-S] cluster is required for FadH activity. However, the *fadH* mutant can also be complemented by human DECR.
Then, we supposed that stresses affecting [Fe-S] cluster biogenesis might impact FadH function. Indeed, we showed that in iron limiting conditions, *E. coli* cannot grow on linoleate, while it still grows on other FAs. However, this defect was not rescued by DECR, suggesting another level of action of iron on FA degradation.
Finally, we found that in a *fadH* mutant, linoleate prevents the use of other FAs. We screened *fad* genes to test if a specific step of the machinery was limiting and identified FadD as the limiting step. In addition, we obtained suppressors which will help us to understand the molecular basis behind the inhibition of FA degradation caused by linoleate in the *fadH* mutant.

Our study highlights the importance of FadH in the presence of different carbon sources or different stress conditions, likely to mimic the gut environment containing complex FA mixture from the diet.

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A61 Virtual screening-based approach to identify novel inhibitors of FtsZ active against *Staphylococcus aureus*

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Infectious diseases are among the top ten causes of death worldwide. In particular, the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.) pathogens show high levels of multi-drug resistance (MDR). To identify novel antibacterial drugs active against MDR ESKAPE pathogens, we targeted the essential divisome proteins by Computer Aided Drug Design (CADD) methods. Thus, a structure-based virtual screening procedure was applied to the *S. aureus* FtsZ structure. Libraries of natural and non-natural compounds were docked to the putative binding site of FtsZ, obtaining a list of 24 compounds.
The compounds were tested for their inhibitory effect against *S. aureus* FtsZ GTPase activity and three of them (3, 8 and 12) showed an IC_{50} of 14.6, 8 and 60 μM, respectively. Since FtsZ cellular function is based also on its polymerization, the sedimentation and the 90°light scattering assays were used to measure polymerization. While the three compounds able to block FtsZ GTPase activity did not affect its polymerization, compound 11 was able to alter FtsZ polymerization in vitro.
The antibacterial activity of the compounds was evaluated against *S. aureus* ATCC25923,

revealing a MIC of 2 µg/ml for compound 11. Time-lapse and confocal fluorescent microscopy were performed to localise FtsZ in the presence of this compound showing that FtsZ polymers were thicker, cells were larger and the fluorescence diffused. The achieved results showed that compound 11 is a promising anti-staphylococcal molecule and further investigations will help to better characterize this FtsZ inhibitor.

A62 Insights on the role and regulation of *M. tuberculosis* Sigma Factor C

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Mycobacterium tuberculosis genome encodes thirteen sigma factors, small subunits of the RNA polymerase that allow rapid adaptation of the bacterium transcriptional landscape, playing a fundamental role in stress-response and pathogenesis. Among them, alternative Sigma Factor C (SigC) is still poorly characterized: its function is seemingly linked to copper uptake and biofilm formation, but its regulon remains undefined. Furthermore, no anti-sigma has been associated to it yet, although the protein Rv0093c represents a suitable candidate.

In order to investigate SigC role in the physiology of *M. tuberculosis*, mutants lacking its structural gene, as well as *rv0093c*, were generated and analyzed in a number of contexts such as different culture conditions, SigC-related gene expression and biofilm development.

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Although no significant differences were found between *M. tuberculosis* wildtype strain and its mutants when cultured in low-copper conditions, a marked sensitivity to high-copper concentrations was detected for the mutant lacking *rv0093c* compared to the wildtype strain. Moreover, genes that had been reported as part of SigC regulon were indeed upregulated in the *rv0093c* KO mutant, supporting the hypothesis of Rv0093c effectively being SigC-specific anti-sigma factor, and refining SigC implication in copper metabolism.

Evidence of physical interaction between the two proteins was also obtained experimentally, by setting up a mycobacterial protein fragment complementation assay, executed in the non-pathogenic model species *Mycobacterium smegmatis*.

A63 The cell envelope biogenesis protein AsmA is essential for outer membrane homeostasis under LPS transport impairment in *Escherichia coli*

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Gram-negative bacteria (GNB) are intrinsically resistant to many antibiotics thanks to the selective permeability properties of an additional outer membrane (OM), which surrounds the cytoplasmic inner membrane (IM) delimiting the aqueous periplasmic space, where the peptidoglycan layer (PG) is embedded. The OM is an asymmetric bilayer, with the inner leaflet comprising glycerophospholipids (GPL) and lipopolysaccharide (LPS) forming the outer leaflet.

Manufacturing a functional OM is fundamental for GNB survival and requires dedicated protein machineries to assemble its components without impairing the barrier function. LPS assembly at

the cell surface relies on the activity of the Lpt transenvelope multiprotein complex comprising, in *Escherichia coli*, seven essential proteins (LptA-G). On the contrary, the molecular mechanisms of GPL transport across the periplasm and assembly in the inner leaflet of the OM have remained elusive till date. Recently, three members of the so-called AsmA-like family of proteins have been involved in GPL trafficking, whereas the other three members (AsmA, YhjG and YicH) have been suggested to work in an independent pathway. Here we provide preliminary proteomics and genetics data pointing to a crucial role for the AsmA protein in OM homeostasis during stress conditions that cause growth arrest, specifically: i) entry in stationary phase and ii) block of LPS transport to the OM due to depletion of the LPS transport protein LptC. Importantly, *lptC* depleted cells bearing *asmA* deletion undergo cell lysis that can be prevented by overexpression of specific PG remodelling enzymes that maintain cell envelope robustness under stress conditions.

A64 Interkingdom cross-talk between symbiotic nitrogen fixing rhizobia and rhizospheric fungi: synergism, antagonism or neutralism in plant growth promotion?

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Aim

In the reconstruction of nitrogen-fixing synthetic communities, a key point is to analyse the presence of biotic relationships between symbiotic nitrogen-fixing rhizobia and the other members of the plant microbiome[1]. Here, as proof-of-concept, we aim at exploring the existence of interactions between rhizobia strains and *Trichoderma* sp. strains, hence synergistic roles of rhizospheric fungi in the symbiotic nitrogen fixation and host plant growth.

Methods

We selected 4 *Sinorhizobium meliloti* strains and 4 *Trichoderma* species. In an experimental scheme of 4 x 4 interactions, we investigated the fungal growth inhibition by dual cultures, the rhizobia growth inhibition and the transcriptomic response elicited by fungal spent media, as well as spent media effects on rhizobia PGP abilities and the effects of the different combinations on the host legume *Medicago sativa*.

Results

Fungal spent media had large and specific x strain specific effect on rhizobia, indicating a general rhizobia genotype x fungal genotype interaction. In particular, a high number of genes was shown to be differentially expressed in rhizobia strains, as well as changes in exopolysaccharide, auxin production and in plant symbiotic phenotypes were identified.

Conclusion

Our results provide a first insight into symbiotic nitrogen-fixing rhizobia and rhizospheric fungi interactions. Given the importance of ensuring more sustainable crop production systems, the dissection of such interactions could contribute the knowledge for a rational use of rhizobia as bioinoculants and development of synthetic communities.

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A65 Whole genome sequencing of *Lactobacillus gasseri* 1A-TV and *in silico* identification of bacteriocins

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Lactobacillus gasseri 1A-TV is a strain with strong antimicrobial activity against a broad range of Gram-positive and Gram-negative pathogens that are responsible for life-threatening infections. As described in our previous study, this effect is exerted by the production of several metabolites yet to be characterized, which could be implemented in novel strategies to fight infection. The aim of this work was to perform whole genome sequencing of *L. gasseri* 1A-TV in order to identify the presence of bacteriocins and evaluate its activity against ESBL and carbapenemase producing *K. pneumoniae*.

Total DNA of *L. gasseri* 1A-TV was sequenced by the Illumina MiSeq platform, assembled by SPAdes v3.14.0 and annotated with RAST. The in-silico prediction of bacteriocins was performed using the antiSMASH 7.0 and BAGEL4 tools. The antimicrobial activity of *L. gasseri* 1A-TV was tested against MDR *K. pneumoniae* by deferred antagonism test using the agar spot test.

The genome of *L. gasseri* 1A-TV amounted to 2,018,898 bp with 34.9% GC. A total of 1,937 putative protein coding sequences, 55 tRNA and 4 rRNA were detected by RAST. Genome mining by BAGEL predicted two bacteriocin biosynthetic gene clusters (BBGCs): BBGC1 contained two class IIc bacteriocins, namely blp-like and gassericin A, while BBGC2 carried the bacteriocin helveticin J belonging to class III. Furthermore, 1A-TV showed strong inhibitory activity against ESBL and carbapenemase producing *K. pneumoniae* by agar spot assays.

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These findings validate the presence of three genes encoding bacteriocins in the genome of *L. gasseri* 1A-TV, which could play an important role in its antimicrobial activity. Moreover, this further confirms the potential of using this strain and its products as alternative strategies to eradicate the threat of antibiotic resistant pathogens.

A66 Dissecting DpaA role for *Escherichia coli* survival to outer membrane stress

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Gram-negative bacteria have a unique cell envelope consisting of a lipopolysaccharide-containing outer membrane (OM) that is covalently linked to the thin layer of peptidoglycan (PG). The OM serves as a barrier against toxic molecules including many antibiotics and allows the cells to survive in many environmental stress conditions. The growth of OM and PG layers needs to be tightly coordinated. Our laboratory recently found that when the OM biogenesis is compromised a PG remodeling program is required to avoid cell lysis. In *Escherichia coli* cells this modification program relies on the activity of LD-transpeptidase family proteins that introduce the non-canonical 3-3 cross-links in the PG layer to restore the mechanical strength and the overall stability of the bacterial cell envelope. Among the member of this family, DpaA is the enzyme that detaches Lpp from PG. Notably, Lpp is the abundant *E. coli* OM lipoprotein that covalently

links the OM to the PG. Previous works of our laboratory have shown that a mutant deleted for *dpaA* undergoes lysis when LPS transport to the OM is blocked. However, the lysis phenotype of the lipopolysaccharide defective *dpaA*-deleted mutant is suppressed by the deletion of *actS* which codes for an activator of amidases, the enzymes that hydrolyze septal PG during cell separation. Our goal is to understand the interplay between *dpaA* and *actS* to better define the physiological role of *dpaA* under envelope stress conditions and the biological meaning of Lpp dynamic attachment to the PG.

Session B - Environmental and industrial microbiology

[Continues from Oral session B. Click here to view the abstracts from B1 to B12]

B13	**Exploring the effects of microplastics on prokaryotic communities**

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Over 80% of the plastic waste produced during the last 70 years is dispersed in the environment, thus representing a global environmental and social issue. Plastic accumulated in the environment undergoes physical, chemical, and biological degradation that enhances its fragmentation into smaller particles, called MicroPlastics (MPs). Microorganisms play key roles in ecosystem functioning, including biogeochemical cycles. Nevertheless, anthropogenic contaminants can affect prokaryotic communities, which are also the major players of xenobiotic degradation. On the other hand, macroscopic plastics can be consumed and biodegraded by insect larvae thanks to the adaptability of their gut microbiome to different foods. Despite the relevance of MPs as ubiquitous pollutant, few studies have addressed their impacts on microbial communities. Moreover, the degradation activity by insect larvae and the effects on the gut microbiome of MPs have not been yet investigated. As part of the multidisciplinary project MEMBRANE (Microplastics Effects on Microbial BiodiveRsity ANd Ecosystem functioning: from knowledge to possible solutions), two experimental systems are currently under investigation in controlled conditions: 1) marine sediments exposed to different (types and dimensions) MPs in microcosm tests, 2) mealworm (*Tenebrio molitor*) larvae fed with macroscopic plastics and/or MPs. The present work will present the experimental designs as well as the preliminary data obtained by Next-Generation Sequencing of 16S rRNA genes on the changes in the composition and diversity of bacterial communities of the two studied experimental systems exposed to MPs.

B14 Inter-disciplinary approaches for studying mulching practices and their influence on soil microbial communities

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Mulching is a widespread agricultural practice aiming at improving crop yield that often involves covering the soil with polyethylene or biodegradable films. The polyethylene used for mulching contains additives, such as phthalates, which are recognized as endocrine disruptors and associated with adverse effects on human health since the gathered pollutants could enter the food webs via soil and crop plants. Moreover, little is known about substances that could be released from biodegradable mulches and potentially endanger biodiversity and agricultural soils. Therefore, there is a need to determine whether polyethylene, biodegradable and other mulches could represent a risk to soil health and food safety. In this work, a small-scale in-field experiment reproducing the strawberry cultivation cycle was set up to investigate the spatio-temporal path of contaminants from mulches to agricultural soil, strawberry plants, and fruits. Preliminary analyses of contaminants contained in mulch films were obtained for the further quantification of these contaminants in soil samples, in plants, and in strawberries. The soil microbial community structure and diversity (bacteria and fungi) are characterized using a metagenomics approach (V3-V4 region of 16s rRNA and ITS1- ITS4 region of ITS amplicons sequencing). Moreover, a smart biomimetic sensor is developed and validated for monitoring the release of contaminants in the soil in a fast and user-friendly way. These interdisciplinary approaches will provide information on the safety of the commercially available mulching materials based on i) potential soil contamination ii) changes in soil microbial community iii) plant health.

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B15 *Camelina sativa* meal hydrolysate as sustainable biomass for the production of carotenoids by *Rhodosporidium toruloides*

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Biorefineries based on residual biomasses are increasingly of industrial interest, to avoid edible resources and improve bioprocesses sustainability. To release sugars from lignocellulosic biomasses the use of enzymes is pivotal, due to their low environmental impact and applicability in different processes. We focused our work on the exploitation of leftovers from *Camelina sativa* oil extraction, called *Camelina* meal, mainly used as animal feed, to produce carotenoids by fermentation of hydrolyzed sugars with *Rhodosporidium toruloides*, a natural carotenogenic yeast. Since most of the market of carotenoids is covered by petrochemical synthesis, there is an increasing quest for molecules of natural origin, considering their applications in the food, feed and cosmetic sectors.

In order to exploit *Camelina* meal, the biomass was saccharified by enzymatic hydrolysis to obtain sugars converted by *R. toruloides* into carotenoids in both Separated Hydrolysis and Fermentation (SHF) or Simultaneous Saccharification and Fermentation (SSF) processes. The

loading of enzymes was reduced to increase economic appeal to the proposed processes. Initial content of total solid was also modulated to improve carotenoids productivity. Possible outcomes of the process are either pure carotenoids or *Camelina* meal enriched in carotenoids, for the feed sector. The process was further tested at the bioreactor level in order to assess the reliability of data from shake flasks fermentations. This work paves the way to the use of other underrated biomass to produce a commodity of interest for several sectors, optimizing the sustainability of each steps of the process, as in the light of principles of bioeconomy.

B16 Identification of New Extremozymes for Biomasses Valorisation through Bioprospecting of Marine Antarctic Microbial Communities

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Microbial communities inhabiting the Antarctic Ocean show extremophilic adaptations conferring interesting properties to their enzymes, which could be exploited for biotechnology and bioremediation. As use of cold- and salt-tolerant enzymes allows to limit costs, reduce contaminations, and minimize pretreatments[1], we recently screened our Antarctic strain collection, comprising 186 morphologically diverse microorganisms isolated from marine biofilms and seawater from Terra Nova Bay (Ross Sea, Antarctica). The screening highlighted proteolytic (52.2%), lipolytic (38.2%), amylolytic (28%), chitinolytic (8.6%) and laccase-like (13.4%) activities amongst the strains. In particular, a new protein (Ant laccase) belonging to the copper resistance system multicopper oxidase family was characterized, produced by seawater *Halomonas* sp. strain M68. Ant laccase oxidizes ABTS and 2,6-dimethoxy phenol, and shows good thermostability, maintaining more than 40% of its maximal activity at 10°C. Furthermore, it is salt- and organic solvent-tolerant, paving the way for its use in harsh conditions[2]. Meanwhile, a chitin deacetylase-like activity was identified in *Acinetobacter* sp. strain c33, isolated from marine biofilm. It proved able to quickly deacetylate para-nitroacetanilide as well as chitosan and colloidal chitin in under 24 hours at 37°C, and was not inhibited by salts. As chitin is currently considered a waste product of the seafood industry, this enzyme could be applied to valorise it by producing higher-value chitosan and chitooligosaccharides under mild conditions[3]. Our efforts show that bioprospecting Antarctic marine microbial biodiversity can lead to the identification of innovative enzymes with promising industrial applications.

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B17 The "Italian white truffle" *Tuber magnatum* Picco and the challenge to identify ascoma-associated bacteria as markers for its geographic traceability

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Tuber magnatum Picco, often known as the "Italian white truffle", is the most expensive of all gourmet truffle species, with a retail price of thousands of euros per kilogram and a trade volume of around 0.9 billion euros per year. High truffle prices have led to several forms of adulteration, including the addition of lower-value truffle species (e.g., *T. borchii*) in place of *T. magnatum*. In addition, since the cost of *T. magnatum* can vary depending on its provenience, even at a local/regional scale, it is likely that ascomata collected from less renowned territories can be sold on the market as the most expensive "Tartufo bianco di Alba". Accordingly, it is of great importance to identify specific markers of *T. magnatum* provenience because this could allow the development of a reliable tracking system with the dual objectives to prevent exposure to fraud in trade and to identify unique features of truffles from different regions. Ascoma-associated bacteria appear to be promising candidates for *T. magnatum* geographic traceability, since there is some evidence that peculiar taxa can be linked to fruiting body provenience.

For this reason, more research should investigate the bacterial communities associated with *T. magnatum*, with the purpose of detecting and identifying microorganisms that could be used as biomarkers of its origin. The availability of high-throughput sequencing technologies that can be applied to large-scale investigations of *T. magnatum* populations could help researchers to identify these biomarkers, in order to develop easy, rapid, and cheap protocols for their detection.

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B18 Biofilm forming bacteria in food processing environments

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Biofilms formed in food processing environments could be a carrier of a wide range of microbial contaminants which can cause adverse effect on food deteriorations as well as compromise the safety of food products.

Tests were performed with 15 S. Enteritidis strains. Bacterial suspension (~1-2 x 10^8 CFU/mL) was inoculated to surface of glass and stainless steel coupons. Bacterial adhesion was provided during the 3h at 25/37°C. Subsequently, suspension was removed and coupons were washed with physiological saline, and submerged in 2 mL of Tryptone Soya Broth (TSB). Coupons were incubated for 48h at 25/37°C. Excess medium and non-adhered cells removed by mild pipetting with 3 mL of saline. Each coupon was placed in tube containing 1 mL of saline peptone solution. Detachments of bacteria were performed exposing tubes with coupons to low energy ultrasound for 3 minutes at 40 kHz, using ultrasound water bath. The number of cells that form biofilm was determined by a standard technique of colony counts and the results were expressed as log CFU/cm^2.

The results imply that adhesion ability varied among tested isolates depending on the tested temperatures and surfaces. Obtained findings showed that that tested isolates produced

significantly more biofilm at 25°C and exhibited a greater propensity to adhere to stainless surfaces, but statistically significant differences were not found. Within this research, the ability of SE isolates to colonize surfaces was demonstrated, particularly at ambient temperatures, which are common in food processing facilities.

Keywords: Salmonella Enteritidis, biofilm, glass surfaces, stainless surfaces.

B19 Biodiversity and metabolic potential of the microbiota thriving in ancient and pristine orthoquartzite subterranean environments

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Caves are subterranean ecosystems characterized by relatively stable temperature and humidity, darkness, and poor supply of organic nutrients. Microorganisms colonizing caves have evolved metabolic processes independent of photosynthesis and adapted to the scarcity and sporadic organic carbon inputs. Cave study has interests in different fields from environmental microbiology to astrobiology and biotechnology. The aim of this work was to investigate the composition, metabolism, and biotechnological potentials of microbial communities and single strains of biofilms and biodeposits from the orthoquartzite caves in the Venezuelan Tepui mountains. These are pristine and ancient subterranean environments hosting outstanding silica speleothems with stromatolite-like formations of microbial origin. Microbial analyses were conducted using cultivation-based approaches (with bacterial mutant construction and screening) and metagenomic analyses both in the laboratory and in situ by setting a temporary laboratory in the cave and optimizing experimental and bioinformatic procedures for limited resources and minimal laboratory settings. Cave microbial communities showed a high diversity of bacteria adapted to moderate acidic conditions and oligotrophy. Novel lineages of Ktedonobacterales, Acidobacteria, and Alphaproteobacteria were found to be dominant in consecutive phases of silica rock alteration. Specific genetic functions and microbial phenotypes were found by analysing metagenome-assembled genomes (MAGs) and cultivated bacterial strains that can be associated with competition strategies (antimicrobial production) and with the development of microbial communities in dark, oligotrophic and silica-based environments.

B20	**Molecular approaches to activate secondary metabolite biosynthesis**

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The search for novel molecules to combat antibiotic-resistant microbes represents an urgent challenge for global health. Secondary metabolites, characterized by low molecular weight, possess complex chemical structures and exhibit a range of biological activities, including antimicrobial, antitumoral, immunomodulatory, etc. Our objectives are to explore pristine environments (i.e., organic farming soil, hydrothermal vents, saltern ponds, activated sludge, and cultural artefacts) to search for bacteria-producing secondary metabolites, and activate cryptic or silent gene clusters devoted to their biosynthesis.

To the latter aim, we intend to overexpress regulatory genes whose corresponding transcription factors could determine the activation of unexpressed biosynthetic gene clusters, leading to the discovery of new bioactive compounds and improving their production. We assembled a collection of 500 bacterial strains, encompassing Gram-positive and Gram-negative microorganisms. We selected 108 Actinomycetes, Gram-positive soil bacteria, as they are the most prolific microbes synthesizing secondary bioactive metabolites.

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We conducted the metabolomic profiling of ten antibiotic-producing bacteria, unravelling metabolites active against Gram-positive multi-resistant strains. Four Actinomycetes were selected to activate secondary metabolites biosynthesis by the introduction of plasmids encoding various classes of transcriptional regulators from model *Streptomyces* strains. The genome of these strains was sequenced, and genome mining analysis through the antiSMASH, a web-based analysis platform, is underway to identify the biosynthetic gene clusters and determine novel bioactive molecules. This work could give more insights into the metabolic potential of novel Actinomycetes.

B21 Hydrogenotrophic metabolisms in the subsurface: insights from natural hydrogen seeps in diverse geological settings

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Hydrogen is a fundamental electron donor in several microbial metabolisms and is considered to be the main energy currency of microbial communities in anaerobic environments. Hydrogen represents one of the keys to a greener energy society. In geological settings, hydrogen can be naturally produced by a variety of processes, including microbial fermentation of organic matter, radiolysis of water, and hydration of iron-rich ultramafic rocks. When hydrogen is released from depth, it travels towards the surface, traversing a large subsurface ecosystem. Microbial communities in the subsurface can use hydrogen as an energy source, coupling its oxidation to the reduction of a variety of different compounds, through a diverse group of enzymes called hydrogenases, catalyzing the oxidation of molecular hydrogen to protons and electrons. These diverse enzymes are characterized by a binuclear Ni-Fe, Fe-Fe or Fe center, as well as multiple FeS centers. Understanding the diversity of hydrogenases in the subsurface and the role of trace elements' availability in controlling their spatial distribution is crucial to quantify the subsurface microbial utilization of molecular hydrogen derived from geological reactions. Here, we will present data on the diversity of hydrogenases from a number of deeply-sourced springs located in diverse convergent and divergent margins worldwide. The results will help to establish the baseline of hydrogenotrophic metabolisms in the subsurface, complementing our knowledge of the microbial influence on hydrogen cycling in various geological settings. Data such as these will improve our understanding of subsurface hydrogen aiding both natural hydrogen exploration and geological hydrogen storage.

B22 Potential use of plant growth-promoting Bacilli as bioremediation in soils contaminated by copper and nickel.

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Due to natural and anthropogenic processes, heavy metal contamination of soil represents a considerable risk for humans and the biosphere, negatively affecting ecosystem health. Traditional contaminated soil remediation technologies are complex processes that reduce soil fertility and are ineffective in treating low-concentration, large-scale heavy metal contamination. As a result, an environmentally friendly and safe strategy to ensure agricultural productivity is required. Among alternative approaches, Plant Growth-Promoting Bacteria (PGPB) are receiving increased interest. PGPB enhance plant growth, as well as protect plants from several biotic and abiotic stresses through a variety of mechanisms. Moreover, these beneficial bacteria create symbiotic relationships with plants and can alleviate the toxicity of heavy metals through metal biosorption, bioaccumulation, redox reaction, mobilization, precipitation, and transformation. In this study, we tested two different consortia of Bacilli PGPB to alleviate copper and nickel contamination in wheat plants. Both consortia can tolerate up to 1000ppm of $CuSO_45H_2O$ and $NiCl_26H_2O$, and the ICP-MS analysis demonstrated the ability of the PGPB to reduce the concentration of metals in media. When inoculated in metal-contaminated soil, both consortia significantly improved the survival of wheat plants', increasing the root and shoot growth and total fresh weight. The consortia were also able to inhibit the growth of wheat plants pathogenic fungi in the presence of heavy metals. One of the two consortia resulted most performed, and they could be proposed as bioinoculant for the metal bioremediation process and as an alternative to chemical pesticides and fertilizers.

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B23 A bacterial consortium for treatment of Fat, Oil, and Grease (FOGs) in wastewater treatment plants

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Fats, oils and greases (FOG), CER 190809, in wastewater create problems including the production of foul odours, the blockage of sewer lines and interference with the proper operation of sewage treatment works. Removal of FOG is thus critically important to ensure that wastewater is disposed of efficiently and economically. In this study, 232 bacterial strains were isolated and screened for the ability to degrade lipids from a specialized treatment plant, where a enhanced FOG degradation activity had been detected. Enrichment cultures on oily substrates, followed by isolation on selective media for lipase producing bacteria, and enzymatic methods were used to screen lipolytic microorganisms. In a second step, the lipolytic bacteria were analyzed using a colorimetric assay to detect the transesterification activity of para-nitrophenyl-palmitate. Four best performing lipid-degrading bacteria were identified by 16SrDNA sequencing, and investigated for application in treatment of lipids-contaminated wastewater, in Sequencing Batch Reactor (SBR) pilot plant. The FOG biodegradation efficiency was evaluated after 10 days using the gravimetric method for quantitative determination of total oily substances. The bacterial strains were Gram negative affiliated to *Serratia*, *Aeromonas*, *Pedobacter* genera. The Bacterial consortium was

able to degrade 76% of FOG in 10 days treatment. The strains are be of great interest at industrial scale to increase the removal of FOG in wastewater treatment plants, and directly in the waste storage tanks at catering establishments, to reduce the polluting load before transfer to the disposal plan. The process of bioaugmentation using the FOG degrading consortium was recently patented (n°812021000056699).

B24	**Isolation and characterization of novel hydrocarbon-oxidizing bacterial strains for the environmental remediation**

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Nowadays, industrialization and urbanization have determined an increase of the demand for petroleum hydrocarbons and, accordingly, of the risk for air, soil and water pollution, because of oil extraction and fuel transport accidents. Therefore, due to the increasing number of hydrocarbon-contaminated sites, it has become essential and a priority to restore these areas through biological remediation technologies. The main purpose of this research was to isolate and characterize novel hydrocarbonoclastic bacterial strains, potentially capable of producing biosurfactants and employable in bioremediation. Accordingly, water and surface sediments were collected from two sampling sites in the municipality of Tramutola (Southern Italy), which are naturally hydrocarbon-contaminated environments.

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Through a combining of molecular and culture-dependent approaches, different bacterial strains were isolated by enrichment cycles in Bushnell-Haas (BH) mineral medium supplemented with diesel fuel as the sole carbon source and characterized from a morphological, genetic (through 16S rRNA-encoding gene sequencing), and metabolic point of view. In addition, they were tested for biosurfactant production through the analysis of emulsifying capacity and emulsion index.

The results obtained from these assays showed that these strains were able to produce emulsions with diesel fuel with high stability over time. These preliminary results pave the way for further investigations aimed at defining in more depth the degradation capacity of the isolated bacteria, and/or of microbial consortia, for the remediation of hydrocarbon-contaminated sites and their ability to produce molecules with a promoter effect for the removal of petroleum hydrocarbons.

B25	**The bacterial communities colonizing five urban caves in Rome, Italy**

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Urban cavities are man-made cavities located in metropolitan areas. While natural caves are well-studied model ecosystems characterized by darkness, physical insulation, and dependence on internal microbial biomass production for energy supply, little is known about the urban cave ecosystems and the microbial communities thriving in these environments. We investigated the bacterial community structure in groundwater, sediment, and biofilm samples from five tuff caves in the metropolitan city of Rome (Italy) underground, all originating from pyroclastic deposits. The physicochemical analysis of the water and sediment showed oligotrophic conditions and

remarkable levels of anthropogenic pollution, with sediments containing 20 mg/kg and 860 mg/kg average concentrations of total hydrocarbons and phosphorus respectively. The 16S rRNA *gene metataxonomic profiling revealed a heterogeneous bacterial population composed of the Proteobacteria*, *Nitrospirae*, *Acidobacteria*, *Firmicutes*, *Actinobacteria*, *Gemmatimonadetes*, *Chlamydiae*, *Planctomycetes*, altogether accounting for 89-94% relative abundance. This phyla distribution was similar for microbiomes from all five caves, with Proteobacteria as the most abundant phylum, followed by *Nitrospirae* and a high percentage of unclassified bacterial lineages. At lower taxonomic level, a remarkable diversity of individual microbial communities was observed, with very few amplicon sequence variants (ASVs) shared by cavities and matrices. *Comamonadaceae* were identified as a biomarker of groundwater ($p<0.05$) by linear discriminant analysis effect size algorithm. Microbial functional groups for the ASVs were inferred from prokaryotic taxa (FAPROTAX) analysis. Our findings demonstrate that urban cavities harbour diverse microbial communities characterized by a high richness of taxa adapted to thrive in polluted and aphotic underground environments.

keywords: Urban caves; Rome; 16S rRNA metataxonomic profiling; Physicochemical analysis; Volcanic rocks

B26 Bacterial Lipopeptides: A New Frontier for Biotechnological Applications

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Biosurfactants are receiving more attention as a potential replacement for synthetic surfactants owing to their advantages, including biodegradability, ecological acceptability and tolerance to extreme conditions. However, they have not yet been widely used in industries because of their low yields and relatively high production and recovery costs.

The present work aims to study the potential of a new hydrocarbonoclastic marine bacterium, Halomonas venusta PHKT, to produce an efficient biosurfactant Bios-PHKT. Biosurfactant production was examined on different carbon sources; using the surface tension measurement and the oil displacement test. Strain PHKT showed its capacity to produce biosurfactants from all tested substrates, in particular glycerol, which is a cheap renewable carbon source, thus minimizing the high production cost. ESI-MS analysis revealed that Bios-PHKT belongs to lipopeptides class. The critical micelle concentration of Bios-PHKT was 125 mg/l. Furthermore, Bios-PHKT showed an interesting stability against a broad range of salinity, temperature and pH. Moreover, Bios-PHKT showed no cytotoxic effect against HEK-293 cells, at concentrations ≤ 125 µg/ml.

Thereafter, crude Bios-PHKT was tested in several biotechnological applications. This lipopeptide showed excellent anti-adhesive and anti-biofilm activities even at low concentrations. Additionally, Bios-PHKT exhibited an interesting anti-proliferative activity against B16 melanoma cell line. Moreover, the evaluation of in vitro and in vivo cicatrization process of Bios PHKT showed that it improved efficiently the percentage of wound closure.

These beneficial biological properties suggest that the crude biosurfactant Bios-PHKT may find significant use in pharmaceutical, cosmetic, or healthcare applications.

Keywords: Lipopeptides, Anti-biofilm, Anti-adhesive, Anti-proliferative, Wound healing

B27 Cyanobacteria as key instruments for a sustainable economy: the role of exopolysaccharides in metal bioremediation

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Cyanobacteria are widespread photoautotrophic microorganisms with interesting physical-chemical properties representing potential instruments to sustain the transition into a circular economy. As an example, cyanobacterial exopolysaccharides can be adopted for metal biosorption and valorized into added-value compounds. We studied the removal of Cu, Ni, and Zn by two halotolerant unicellular strains CE4 and 16Som2 as well as the possible valorization of the metallic-organic materials harvested at the end of the biosorption process. The different role in metal uptake by released polysaccharides (RPS) and the cells at varying stages of the growth curve was investigated while binding mechanisms and structural properties of the obtained materials were studied through SEM/EDX, ICP-OES, and EXAFS techniques. Even if the absolute removal generally increased with time, the specific uptake decreased. Indeed, the highest Cu removal (50%) was shown by 16Som2-RPS after 18 days of growth. While the highest specific uptake was shown by CE4-cells after 7 days (0.47 mmol g/dry weight, 1.67 mmol g/carbohydrate). The simultaneous presence of the three metals had a positive effect on Cu uptake and a negative effect on Ni and Zn uptake. Additionally, we found that Ni and Zn weren't adsorbed on the surface of the cells, while Cu was adsorbed on the surface of the cells and also accumulated inside. Preliminary studies for the valorization of the obtained materials revealed promising results for catalytic activity.

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B28 GeoMosaic: A flexible metagenomic pipeline combining biological and geochemical data to outline biosphere and geosphere interactions

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Introduction

The interaction between the biosphere and geosphere is essential for their co-evolution and has contributed to keeping our planet habitable for more than 4 billion years. Metagenomic studies of the environmental microbial communities are essential to describe them and their adaptations necessary to colonize such environmental conditions. Integrating this information, along with the geochemical and geological context is fundamental to outlining a complete overview of the bio-geo interaction. Here we present GeoMosaic, a flexible (work in progress) metagenomics pipeline combining biological and environmental, and geochemical data.

Materials and Methods

It integrates different stream outcomes as reads-, assembly- and MAGs-based analysis to extract and optimize the data knowledge. Each stream contains different modules (e.g. pre-processing, assembly, binning, etc), in which are implemented one or multiple methods to perform the respective task. The designed modularity allows bioinformatic users to complete or update the workflow with new analysis modules, to improve the comprehensive range of analysis topics.

Results

GeoMosaic is a publicly available pipeline, developed to be easy to use by choosing/ignoring both modules and methods for each task. The major input for the users is an Excel tabular file describing the name of the sequencing that reads R1 and R2 and the corresponding sample name.

Discussion and conclusions

Metagenomics is a widely used technique to study the taxonomic composition and metabolic function of microbial communities. Using such sequencing data, the GeoMosaic workflow provides assimilated results of metagenomics and geochemistry steps in ready-to-use data and plots for downstream interpretations.

B29	**Fermented bread wastes for poly-β-hydroxybutyrate (PHB) production by purple non-sulfur bacteria**

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Worldwide plastic production is rising every year. Nowadays, plastics are mainly derived from petrochemical industries and are responsible for a variety of growing environmental issues including the emission of greenhouse gases, accumulation in terrestrial and marine habitats, and pollution. Bioplastics are biodegradable and made from renewable sources or biocompatible, representing an attractive alternative to conventional petroleum-derived plastics. Since the industrial expenses to produce bioplastics are much higher than for petroleum-derived plastics, one option to pursue would be to use agri-food wastes for bioplastic production.

Poly-β-hydroxybutyrate (PHB) is the most well-studied member of the polyhydroxyalkanoates (PHAs), which is a family of biodegradable intracellular polyesters produced by several bacteria. Recently, PHA production from various biowastes is receiving increasing attention for industrial-scale production; in particular, bread wastes, containing an elevated percentage of carbohydrates, represent a suitable source of nutrients for microorganisms.

The aim of this work was to use bread wastes as feedstock for a sustainable production of the biopolymer PHB. Fermented bread broth, obtained from previous lactic fermentation of bread wastes, was used for photofermentation by eight strains of Purple Non-Sulfur Bacteria (PNSB). The strain with the highest PHB production and growth was *Rhodobacter sphaeroides* strain Pisa 7, reporting 44.50 % of PHB/cell weight (w/w). The lowest PHB yields were obtained with *Rhodopseudomonas palustris* species. *R. sphaeroides* Pisa 7 was chosen to scale up the photofermentation into a photobioreactor of 5 L. This study offers a promising contribution to a circular economy, converting bread wastes into a biopolymer using a microbe-based system.

B30 Environmentally friendly textile pigments: implementation of genome mining-based approach for the identification of new anthraquinone producers

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It is estimated that 200,000 tons of synthetic pigments are lost in the effluents of the textile industry each year. Most of these dyes escape conventional wastewater treatments, resulting in adverse effects on the environment, such as ecotoxicity, carcinogenicity, and water turbidity.

Pigments of microbial origin have often been proposed as a safer alternative to synthetic pigments and the bio discovery of new pigment-producing strains has become strategic for a more sustainable textile supply chain. In this context, our study focused on the identification of new anthraquinone-pigment producers, using a predictive genome mining-based approach. In particular, we queried AntiSmash and retrieved predicted polyketide synthase biosynthetic gene clusters (BGCs) for anthraquinone biosynthesis and evaluated the industrial applicability of the respective strains, using WIPO and BacDive, yielding a list of 37 potential new anthraquinone producers.

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We chose candidate strains to evaluate the actual anthraquinone production. To this aim, aerobic batch fermentation processes were set up in 1-L stirred tank bioreactors. Cells were pelleted and extracted with dichloromethane, chloroform, and ethyl acetate. ^{1}H nuclear magnetic resonance analysis followed and revealed signals related to aromatic protons, consistent with the presence of anthraquinones.

In conclusion, by applying a predictive computational approach, we were able to identify new potential anthraquinone-pigment producers. We are investigating strain growth and production processes for the selected strains, performing chemical characterization and evaluating toxicological of the target pigment, to assess the actual feasibility of production scale-up to pilot-scale.

B31 Biodegradability of model polyesters for the valorization of diary wastes: a circular economy perspective

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Cheese whey (CW) is the main by-product of the Italian dairy industry and is mainly composed of protein and lactose. While CW proteins are marketed as a dietary supplement, lactose is still little exploited, and lactose-rich CW permeate (CWP) is considered a secondary by-product with a still

relatively undeveloped market.

In our project, lactose-derived sugars, *i.e.*, glucose and galactose, were chemically modified to obtain monomers that can be used for the synthesis of new bio-based polyesters with biodegradable and biocompatible features. Evaluation of the properties of the sugar monomers suggests that they are biocompatible.

Before assessing the biodegradability and biocompatibility of new bio-polymers, the ability of bacteria belonging to the genus *Rhodococcus* to degrade polyesters was preliminary tested using polycaprolactone (PCL), a slowly biodegradable aliphatic polyester. The metabolic potential of *Rhodococcus* bacteria has been investigated by means of growth and enzymatic assays and correlated with genome analysis to identify enzymes involved in PCL degradation.

Overall, our results suggest that *Rhodococcus* bacteria can be considered as a model for the evaluation of the biodegradability of sugar-based polyesters deriving from the valorization of CWP. Moreover, *Rhodococcus* biotechnological potential emerged also from the mining of the genomic features revealing the possibility to deeper investigate their enzymatic asset for polyester hydrolysis.

This work has been supported by Fondazione Cariplo, grant n° 2020-0838.

B32	**High density polyethylene (HDPE) biodegradation by the fungus *Cladosporium halotolerans***

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Polyethylene is composed of ethylene monomers ($CH_2 = CH_2$) and it is the most common polymer used in the plastic industry. HDPE is mainly composed of linear polyethylene polymers producing a narrow, dense and organized structure. Since Polyethylene is a hydrophobic and high molecular weight synthetic polymer, it is very difficult to biodegrade. To increase polyethylene bio-degradability it is important to find microorganisms that improve the PE solubility and/or reduce the length of its polymeric chain by oxidation. The aim of our study was to isolate new PE-degrading microorganisms from the *Galleria mellonella* larvae gut content and identify their potential PE-degrading mechanism. Here, we describe a new strain of *Cladosporium halotolerans* able to interact with plastic material through its hyphae and to produce enzymes involved in PE degradation.

B33 Analysis of the microbial colonization of biochar used as alternative of activated carbons for sustainable treatment of contaminated waters

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Biochar is an adsorbent carbonaceous material obtained from the pyrolysis of different types of biomasses, like agricultural wastes, that holds promise for low-cost wastewater treatment as an alternative to activated carbon (AC). Bacteria can colonize the filter surface forming biofilms and contributing to filtration capacity, but the mechanisms of their interaction with biochar surface and the communities developed on this substrate are largely unexplored. Our work is aimed at investigating i) the metabolic activity, composition and dynamics of the microbial communities colonizing experimental filters (AC and biochar) applied in different water treatment plants, and ii) studying the possibility to recover filters collected from the plants at the end of their lifecycle by treating them with synthetic microbial consortia or adapted communities. Sequencing data showed that microbial communities' composition differed depending on the treatment plant under analysis and the contamination present. Microbial communities colonizing the filters showed high abundance of specific bacterial taxa associated with biofilm growth, environmental stress resistance and organic contaminant biodegradation. Richness and diversity indexes were similar between AC and biochar applied to the same treatment plant and were typically higher for the communities colonizing filters as compared to waters. Applying synthetic consortia for the regeneration of exhausted filters we observed the adhesion of inoculated bacteria increasing the total biomass on filters for possible nutritional advantage and/or cell adsorption phenomena. GC-MS analyses of the treated filters showed the removal of contaminants providing first indications on the possibility to utilize biological treatment to re-cycle/-utilize end-life filters.

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B34 An innovative highly effective microbicidal coating for high-touch surfaces

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The transmission of pathogens through the contamination of surfaces and fomites represents one of the main modes of indirect transmission. In this context, super crowded places such as schools, airports, hospitals, nursing and hospice, require more control.

In fact, surfaces and objects can have a high microbial load, including pathogenic microorganisms that, coming into contact with individuals with a deficient immune system, may represent a serious risk.

At present, disinfection of environments and objects as well as hand hygiene represent the main means of preventing the indirect transmission.

However, disinfection of public places can be questionable being related to cleaning time as well as meticulousness of the operator and it is difficult to control especially in public places.

This highlights the need to develop technologies that have the ability to reduce or eliminate the microbial load on high contact surfaces.

In this context, we present a biocompatible, economical and not toxic antibacterial coating whose invention is owned by the University of Cagliari (PCT/IB2020/055621), it has demonstrated 100% antibacterial activity on the microorganisms tested representing an innovative and valid approach in order to reduce the microbial load on contaminated surfaces exposed to the environment and for medical devices and high-touch objects.

B35 The Great Gobi A strictly protected area: molecular and phenotypic characterization of bacterial communities isolated from soil samples of different oasis

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Soil microorganisms are fundamental components of ecosystems services since they regulate the various biogeochemical cycles by contributing to plant nutrition and soil health. The diversity of soil microbial communities is influenced by several factors, including soil pH, climate, and organic carbon availability. In desert ecosystems, the harsh condition constitutes the main factors limiting the growth and biological activity of microorganisms and understanding how microbial communities adapt to environmental stresses is critical for interpreting ecological patterns and microbial diversity.

Great Gobi A, a strictly protected and intact wild area of the Mongolian desert, represents an intriguing model to study the bacterial community. In this work were characterized the cultivable and total bacterial community of five soil sample from each four different oasis of the Great Gobi A. Preliminary results shown a high variability of microbial diversity in the various sampling of each and between the oases. The different amount the organic carbon in the various soil samples of each and different oasis is one of the key factors that drives this different microbial diversity. Among the bacterial isolates collection, several isolates belonging to Arthrobacter genus presented interesting environmental and clinical aspects. 13 isolates were tested versus environmental and human pathogens and different concentration of antibiotics, heavy metals and salinity. An interesting result was obtained from antagonistic test of pathogen and the growth at different levels of NaCl in the medium. Additional factors will be evaluated with the aim to understand the drivers that shape the microbial community in this unique habitat.

B36 Biodiversity and metagenomic analysis of cold-adapted microbial communities from Cenote Abyss (Dolomites, Italy)

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Cenote Abyss cave, located at 2940 m a.s.l. in the Regional Park of Fanes, Sennes and Braises, is an impressive carbonatic ice cave of 285 m deep, which hosts one of the most voluminous cave glaciers of the Dolomites. It is a remote and oligotrophic environment that represents an excellent accessible model system for understanding fundamental microbe-mineral interactions contributing to cold subsurface biosphere. Moreover, the huge ice deposits identified within this cave are of great interests for studying the paleoclimate and climate evolution of the Dolomites during the last thousands of years, providing insights into the recent melting of the cave glacier. In this context, microbial life at the cryosphere represents a unique archive of slow biogeochemical processes in ancient and chilly environmental conditions. In this study, ice samples were taken at different depths within the Cenote Abyss, and the microbial communities present were characterized at the taxonomy and functional levels by performing metabarcoding and metagenomic sequencing analyses. The 16S rRNA gene sequencing analysis showed that the microbial communities of Cenote Abyss were dominated by members of *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* well adapted to extreme temperatures and possibly involved in different steps of the nitrogen cycle in accordance with the cave depth. Metagenomic analysis revealed the presence of several genes involved in various metabolic processes involved in the nitrogen geochemical cycle such as ammonium oxidation to nitrate, denitrification, assimilatory and dissimilatory reduction of nitrate and nitrogen fixation, as well as numerous genes associated with the primary metabolism of carbon.

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B37 The abysso-hadal prokaryotic communities of the Kuril Kamchatka Trench: a metabarcoding analysis

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Keywords: Kuril Kamchatka Trench, abysso-hadal prokaryotic communities, benthic boundary layer, metabarcoding, Kurambio II.

Located in the NW Pacific Ocean, the Kuril–Kamchatka Trench (KKT) is among the seven deepest trenches (depth 9604m). Being the microbial diversity of this deep-sea environment practically unknown, this work was aimed at getting the first in-depth investigation of its abysso-hadal prokaryotic communities. Eighteen samples for the metabarcoding (16S rDNA V5–V6 regions)

were collected in various abysso-hadal areas within the KKT by the Research Vessel *Sonne* (Germany), during the KuramBio II expedition. Samples, collected from different trench sites (depth 5146-9540m), were taken from the water overlaying the cores of a multicorer and represented the benthic boundary layer. *Proteobacteria* (56.1-74.5%), *Bacteroidetes* (6.5-19.1%), and *Actinobacteria* (0.9-16.1%) were the most represented bacterial phyla, whereas *Thaumarchaeota* (52.9-91.1%) was the most abundant archaeal phylum. The most abundant microorganisms were chemolithotrophic archaea and heterotrophic bacteria, which did not show a distinctive zonation being detected at all depths. The archaeal diversity was highly represented by the ammonia-oxidizing *Nitrosopumilus*, while the potential hydrocarbon-degrading bacteria *Acinetobacter, Zhongshania*, and *Colwellia* were the main bacterial genera. The α-diversity analysis evidenced that the communities were characterised by low evenness (high Gini index values, >0.9). The β-diversity analysis indicated that depth significantly affected the prokaryotic communities' structure. The co-occurrence network showed seven prokaryotic groups that covaried across the trench abysso-hadal area. The main group included most abundant archaeal and bacterial OTUs (*Nitrosopumilus* OTUA2 and OTUA1; *Acinetobacter* OTUB1), which were ubiquitous across the trench. The current study evidenced that this extreme environment harboured communities that differ from those of other marine trenches.

B38 Multi-omics unveil biomolecular aspects involved in plant-growth promoting activity on *Solanum lycopersicum* by the actinobacteria *Streptomyces violaceoruber* and *Kocuria rhizophila*

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Biofertilizers based on plant growth-promoting bacteria (PGPB) represent an eco-friendly alternative to reduce the environmental pollution due to chemical fertilizers, preserving human health and biodiversity[1]. PGPB exert beneficial effects on plant by improving nutrition uptake and abiotic/biotic stress tolerance[1]. The development of innovative PGPB-based biofertilizers is among the main goals of the BIAS project[2]. Indeed, different putative PGPB were assayed for *i*) indoleacetic acid production, *ii*) organic and inorganic phosphate solubilization, *iii*) saline and drought stresses tolerance, and *iv*) nitrogen fixation. Among the most interesting strains, two actinobacteria, *Streptomyces violaceoruber* and *Kocuria rhizophila*, were characterized for their secreted and cellular metabolome[3]. To confirm their PGPB beneficial role, these strains were inoculated into *Solanum lycopersicum* tomato cultures performed using different experimental systems, spanning from filter paper in Petri discs[3] to controlled conditions and open field, using

seed imbibition possibly coupled with fertigation strategies. Both PGPB strains stimulated growth and development of tomato plants, mainly related to rooting, flowering and fruiting improvement. As revealed by integrating metabolomics, epigenetics, transcriptomics and proteomics, the PGPB effects are linked to a global regulation of plant gene expression related to different cellular processes, such as nitrogen and energy metabolism. Furthermore, PGPB-dependent microbiota structure changes in tomato bulk soils were also revealed in open field experiments. Thus, these results increase the knowledge on actinobacteria-plant interactions and promote the use of these strains to develop novel biofertilizers in the frame of a sustainable agriculture.

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[3]https://doi.org/10.3390/metabo13030374

B39 Study of two different mammalian cell lines *in vitro* transfected with an mRNA vaccine model encoding for enhanced Green Fluorescent Protein (eGFP)

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In the last years the interest in mRNA vaccine technology has raised. In this project, an mRNA encoding the eGFP was used as a vaccine model by transfecting it into two cell lines. Protein expression and cell viability were assessed at different time points. A DNA template, encoding the eGFP, was used for mRNA production by *in vitro* transcription reaction. Two mammalian cell lines (HeLa and C2C12) were transfected with a complex of mRNA and lipofectamines. Transfected cells were analysed by flow cytometry and fluorescence microscopy. Cell viability was assessed by annexin V-propidium iodide staining. Expression of eGFP was already detected after 1 hour in both cell lines. HeLa cells reached the protein expression peak earlier (6h), compared to C2C12 (12h), and both lines showed a decrease at 48h. Furthermore, after 12h of incubation, detached cells were observed only in transfected samples, and not in control ones. Staining with annexin V and propidium iodide confirmed the cell death for transfected cells. Mimicking i*n vivo* conditions, phagocytosis assays were performed with macrophage-like induced THP-1 cell line showing that only transfected cells and not untreated ones were efficiently engulfed. In this work, an mRNA vaccine model was developed, mammalian cells were successfully transfected, and protein expression was detected at different time points. In our model, we observed cell death of 12h transfected cells and demonstrated that only these cells were efficiently engulfed by macrophage-like cells. This suggested us to investigate phagocytes gene expression after engulfment. Transcriptomic analyses are ongoing.

B40	**New insight into the microbiome composition of two Mediterranean coral species**

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In the last 15 years, coral associated microbiome emerged as an extremely important research field for its key role in the health-status of corals and their potential response to diseases and environmental changes. Despite these evidences, nowadays our knowledge of microbial communities associated with corals remains remarkably scarce, especially in the Mediterranean area, due to the novelty of this topic and the difficulties related to the analysis protocols.

In this context, the present study aimed to describe and compare for the first time the microbiome associated with three species of scleractinian corals: two from the Mediterranean Sea (*Cladocora caespitosa* and *Madracis pharensis*) and one from the Maldives (*Herpolitha limax*).

Taxonomic analyses were conducted by Illumina sequencing of the V5-V6 16S rRNA hypervariable regions; moreover Non-metric Multidimensional Scaling analysis was conducted to examine the beta-diversity relationship between the Mediterranean and Maldivian species. From relative abundance data, calculated on the 19 most present families, it was possible to infer the existence of two distinct microbiomes, one on the Mediterranean coral specimens (mainly Comamonadaceae) and the other one on *Herpolitha limax* individuals (mainly Burkholderiaceae and Nocardiaceae).

Further studies are needed to better understand how the different biotic and abiotic factors shape microbiome composition; this information could be crucial to define the microbiome's role in coral survival, especially in the modern context of global climate change. Furthermore, these findings could also be used to improve the main mitigation tools currently used to cope with the problem, such as coral restoration.

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B41	**Could *Pontimonas* harbor halophilic members able to withstand very broad salinity variations? An extensive metabarcoding case of study (The Saline di Tarquinia, Italy)**

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The genus *Pontimonas* (*Actinobacteria*) is currently described as including only one species (*P. salivibrio*) of a slightly halophilic marine bacterium. Its type strain, *P. salivibrio* CL-TW6^T, was isolated from a Korean solar saltern pond having salinity slightly higher than the seawater. Although some works revealed its presence in some other hypersaline environments, information on the habitat preference of this genus is still scant. This work investigated *Pontimonas'* presence in different ponds of the "Saline di Tarquinia" marine saltern (North Tyrrhenian Sea, NW of Rome, Italy) and the nearby sea. The two-year metabarcoding survey revealed its constant presence along the ponds, which established the saltern salinity gradient, and in a distinct nearby basin with permanent hypersaline conditions (BSB). *Pontimonas* was higher in the ponds than in the sea,

whereas it had similar abundances in the sea and in the BSB. Its representative OTUs showed significant trends according to different parameters along the salinity gradient. OTU1 abundance increased along the salinity gradient, with decreasing water temperatures and increasing rainfalls, and it showed a maximum in January; OTU2 increased with increasing BOD_5 and it showed the highest abundances in the period August-October; finally, OTU 3194 increased according to decreasing salinities. In BSB, a significant variation was shown in relation to the seasonality by OTU 3194, which started increasing in spring to reach a maximum in summer. The results of this study suggest that *Pontimonas* could easily settle in hypersaline habitats, having also broad euryhaline representatives, and it might include possible extreme halophilic members.

B42 The effects of plant-growth promoting actinobacteria on *Origanum vulgare* growth and bioactive molecule production

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Plant growth promoting (PGP) bacteria are naturally occurring in plant crop soil and rhizosphere. They are increasing attention for their possible use as biofertilizers, since PGP bacteria exert beneficial effects on plant growth and development by improving, as examples, nutrition uptake, abiotic and biotic stress tolerance in plants[1]. Among the PGP bacteria, the actinobacteria are considered very promising due to their metabolic versatility, bioactive metabolites production and drought resistance. Sicily, like most semi-arid Southern European regions, hosts drought-resistant plants such as aromatics, with a diversified microbiota and high nutritional value, due to the production of valuable bioactive molecules. In this context, the two PGP actinobacteria *Kocuria rhizophila* and *Streptomyces violaceoruber* have been investigated for their possible effects on *Origanum vulgare* growth and bioactive molecule production. In particular, *O. vulgare* cultures were grown in pots and, subsequently, inoculated with actinobacterial cultures or mixtures thereof, using water, bacterial growth medium and a commercial microbial mix biofertilizer as control conditions, respectively. In this regard, the inoculum contribution on the composition of the soil microbiota has been also investigated by mean of culture-dependent approaches to reveal the presence of actinobacteria. The plants were then transplanted into an open field and grown until flowering. Preliminary results showed that PGP bacterial treatments exerted significant effects on selected morpho-physiological parameters like plant height. In addition, leaves and flowers were sampled to perform solid phase microextraction (SPME) analyses to reveal possible qualitative and quantitative changes on plant volatile organic compounds that could be ascribed to plant-actinobacteria interaction.

Reference:

[1]http://dx.doi.org/10.3390/metabo13030374

B43 Colistin and clofoctol have synergistic activity against colistin-resistant Gram-negative pulmonary pathogens

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Colistin is an antibiotic active against most of the Gram-negative pathogens. Although the toxicity of this antibiotic is a worry due to its limited therapeutic range, it has been used in recent years as a last-line defence to treat infections by Gram-negative pathogens when no other treatment options are available. However, resistance to colistin has inevitably occurred among clinical isolates, making the research for colistin adjuvants extremely important. Clofoctol is an FDA-approved synthetic antibiotic active against Gram-positive bacteria, with well-established pharmacological characteristics such as low toxicity and high airway tropism. Interestingly, clofoctol has been shown to have a wide range of biological activities and has been suggested for treating various obstructive lung diseases, including asthma, lung cancer, and SARS-CoV-2 infection. In this study, the activity of clofoctol as a colistin adjuvant was investigated against three major Gram-negative pulmonary pathogens belonging to the ESKAPE group (*i.e.*, *Pseudomonas aeruginosa*, K*lebsiella pneumoniae* and *Acinetobacter baumannii*). The efficacy of the colistin-clofoctol combination was tested in one colistin-susceptible and three colistin-resistant strains of each species by performing MIC, MBC, and time-kill assays. Our results showed that clofoctol enhances the bactericidal effect of colistin in all strains tested and restores colistin susceptibility in almost all colistin-resistant isolates. These results encourage the development of colistin-clofoctol formulations for the therapy of hard-to-treat airway infections caused by Gram-negative pulmonary pathogens. Moreover, mutants resistant to the colistin-clofoctol combination have recently been isolated in order to understand the mechanisms involved in the synergistic activity of the combination.

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B44 First insights into the biodiversity and metabolic potential of microorganisms colonizing the hydrothermal cave system of Monte Kronio

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Caves are subterranean environments that host microbial communities able to thrive under oligotrophic conditions and interact with the cave substrate through a wide range of metabolic activities. These include both the synthesis of secondary metabolites involved in cooperation and competition mechanisms (e.g. antimicrobials, siderophores, hydrolytic enzymes) as well as the secretion of organic acids involved in the dissolution and deposition of minerals. The bioprospecting of the cave microbiome might result in the discovery of new metabolic activities useful for biotechnological applications and can provide insights into the ecology of the cave environment. Here we investigate the biodiversity and metabolic potential of microbial communities present in the hydrothermal Cucchiara cave, located in Monte Kronio (Sicily). In particular, we analysed

rock encrustations and biofilms whose colour (pink, yellow, white, red) seemed to be correlated to the temperature gradient that characterized the cave. The microbial analyses were conducted by using MinION sequencing to analyse the entire microbial communities and by culturable-based approaches. The most represented taxa belonged to the bacterial phyla *Actinobacteriota*, *Planctomycetota*, *Methylobirabilota*, *Proteobacteria*, *Acidobacteriota* as well as the archeal phyla *Nanoarcheota* and *Aenigmarcheota*. Some of these taxa appeared to be differently represented in the diverse collected samples depending on the temperature and sample colour. Among the 47 microbial strains that were isolated, 41 strains showed specific enzymatic activities involved in the hydrolysis of complex polymers, urea and other compounds. Additionally, six isolates inhibited the growth of different human pathogens indicating their capabilities to produce antimicrobial compounds for biotechnological applications.

B45 Comprehensive identification and characterization of the S-layer homology domain (SLH): results of a bioinformatic survey in more than 6300 Bacteria genic sequences

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The outermost portion of many prokaryotes is covered by the S-layer, a proteinaceous crystalline array that seems to be a vestigial structure needed to survive in the ancestral Earth environment. Its biosynthesis is a costly process, which therefore must still provide an advantage that justifies its modern production. Apart from the S-layer homology domain (SLH), S-layer proteins show essentially no sequence homology and this has been a main problem when investigating their role. Hence, although some structural studies have been performed, little is known about the S-layer presence in Bacteria and its functional significance nowadays.

The aim of this study was to expand the knowledge about SLH diversity in Bacteria genomes. Using the 24 SLH-containing proteins available in InterPro database, we build a set of consensus sequences that we used to search SLH motifs in more than 6300 Bacteria genic sequences. This bioinformatic pipeline led to the identification of 16303 hits in 429 Bacteria species, of which only the 6% was previously studied for the S-layer. The validation and characterization of the identified hits showed that the different Bacteria species have from 1 to 133 SLH motifs, as found both in chromosomes and plasmids. The SLH phylogenetic analysis allowed to assess whether microorganisms with a related SLH sequence share the adaptation to a particular environment. Overall, the results obtained will allow to verify in vivo whether the S-layer is formed or not according to the environment and its changes, shedding light on its modern role in the different natural settings.

B46 Microbial chitosan production from digestate-based liquid growth medium

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Several technical elements make the fungal route of production of chitosan an exciting alternative to the conventional extraction approaches from the shellfish residues. In fact, the better quality of chitin and chitosan present in the fungal cell wall and the lower environmental impact of their extraction process from fungal sources compared to that from crustacean waste, boost recent research to mycobiotechnology, relying on fungal strains able to produce chitosan directly in their cell wall, possibly using wastes as production medium.

The present study assesses the possibility of using a digestate-based liquid growth medium for microbial chitosan production. To this end, a digestate arising from an anaerobic process underwent various chemical pretreatments to yield a sugar-enriched liquid phase was tested for its ability to support growth and chitosan production of 17 selected fungal strains. A first scale transfer to bioreactor (STR) has been studied for the best producer strains of both *Absidia blakesleeana* and *Rhizopus oryzae*. To the best of our knowledge, this is the first study claiming the use of a digestate-based liquid medium for fungal chitosan production.

B47 Bacterial biofilms on biopolymeric sorbent supports for environmental bioremediation

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Bioremediation encompasses a broad range of environmental biotechnology, which require multidisciplinary approaches through implementation of innovative tools to the natural biological process occurring in soil, water and air. Immobilization of hydrocarbon-degrading microorganisms on biodegradable sorbent supports significantly promotes bioremediation processes. Recently ecofriendly, low cost bioremediation devices based on polylactic acid (PLA) and polycaprolactone (PCL) membranes hosting a biodegrading bacterial biofilms were obtained[1]. This work investigates the higher effectiveness of immobilizing hydrocarbon-degrading bacteria compared to that of planktonic cells. Soil hydrocarbon (HC) degrading Actinobacteria *Nocardia cyriacigeorgica* strain SoB, *Gordonia amicalis strain* SoCg[2], and the marine hydrocarbonoclastic *Alcanivorax borkumensis* strain AU3-AA-7[3] were immobilized on PLA and PCL membranes and tested on hexadecane. The capacity of adhesion and proliferation of these biodegrading biofilms within the biopolymers were evaluated at various time points (5, 10, 15, and 30 incubation days) using scanning electron microscopy (SEM). The SEM images revealed that PLA and PCL nanofibers were nearly completely covered by a complex three-dimensional bacterial film for all tested strains. Quantification of total biomass (estimated as total dsDNA) confirmed biofilm growth up to 30 days of incubation. Crude oil biodegradation ability of biofilms-membranes systems, assessed

by Gas Chromatography-FID analysis, demonstrated the removal of over 60% of the oil after 5 days of incubation, outperforming free-living bacteria by 24%. Viable plate counts showed that bacterial biofilms adsorbed on biopolymers were still viable after 30 days, indicating their potential for long-term applications.

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B48 Gastroduodenal *in vitro* digestion of Linezolid Resistant Enterococci (LRE) isolated from intensive swine farm.

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Enterococcus faecalis is part of the human and animal grastrointestinal microbiomes. It is able to acquire and transfer antibiotic resistance genes (ARGs). Linezolid resistance, encoded on mobile genetic elements, causes great concern since this molecule is a last resort antibiotic to treat antibiotic resistant enterococcal infections. Specifically, Linezolid Resistant Enterococci (LRE) may exist and spread within intensive swine farms, posing a risk of transmission to consumers through the food chain (*i.e.*, by consumption of salami and raw sausages). Furthermore, enterococci withstand the unfavourable and stressful conditions such as human digestion, reaching the gut in a viable form and hence able to spread ARGs through horizontal gene transfer.

This study aims to investigate the effects of an "*in vitro*" digestion on Linezolid resistant *E. faecalis* strains (n=3) isolated from a swine farm. The strains are positive for the Linezolid resistance gene *optr*A, carried on mobile genetic elements. The obtained results underline that: (i) the digestive stress decreases the number of bacteria; (ii) surviving bacteria maintain the expression of Linezolid resistance; (iii) after the "*in vitro*" digestion the strains can spread Linezolid resistance genes through conjugation at a similar frequency to the undigested strains. Overall, these results demonstrate that LRE, isolated from food-producing animals, could pose a serious safety risk if transmitted to humans.

B49 Cryoconite bacterial community metabolic activity

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Glaciers are ecosystems that host active and rich bacterial communities. They gather mainly in cryoconite holes, small pits on the glacier surface filled with meltwater and sediment (i.e. the cryoconite) on the bottom. These holes are hot spots of biodiversity on glaciers and they host not only bacteria but also algae, metazoans, fungi and viruses. The bacterial community has a pivotal role in the subsisting trophic interactions thanks to its metabolic versatility. With this work, we describe both the active and the total bacterial community of cryoconite from Forni Glacier (Italian Alps) through 16S rRNA and rDNA sequencing. Results showed that the active fraction is only a small part of the total community and it is mainly composed of anaerobic taxa, consistent with the fact that the cryoconite layer is mostly anoxic. Shotgun metatranscriptomic was also performed on a single cryoconite hole at four different times along the same day. Results revealed different patterns of expression throughout the day that can be explained by the different intensities of the incoming solar radiation. Photoinhibition was observed when the solar radiation was the highest, concomitantly with a still subsisting CO_2 fixation likely due to non-phototrophs. The high proportion of H_2-consuming bacteria and hydrogenase transcription suggest that hydrogen oxidation may be used as an alternative reducing power to photosynthesis. Overall, this work shows that DNA sequencing misses an important fraction of the active community, therefore combining DNA and RNA sequencing may provide more insights into the actual role of bacteria in cryoconite holes.

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B50 Unlocking the Potential of *Kitasatospora purpeofusca*: A Promising Rare Actinomycete for Biotic Transformation of Selenite

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The utilization of selenium in industrial applications, alongside the improper disposal of selenium derivatives, determined a concerning accumulation of these compounds in the environment. Among the various selenium species, oxyanion selenite is of high concern due to its high solubility, bioavailability, and toxicity. Furthermore, as a rare Earth element, selenium faces the imminent threat of depletion, triggering potentially significant economic and technological repercussions. Therefore, it is important to recycle and recover selenium from selenium-containing waste. In

the pursuit of sustainable practices aligned with the principles of the circular economy, microbial biotransformation of selenite into less harmful selenium species emerges as the most promising strategy. While actinomycetes are recognized as invaluable players in environmental biotechnology, their involvement in selenite biotransformation remains largely unexplored, primarily due to a limited understanding of oxyanion bioprocessing. In this study, *Kitasatospora purpeofusca*, a rare actinomycete isolated from agricultural soil, features a series of striking cell responses when dealing with selenite, including morphological changes, cell membrane alterations, an outburst of oxidative stress, the participation of thiol-based chemistry for selenite processing, and the generation of selenium nanomaterials consequently to selenite transformation. Moreover, findings on a laboratory-evolved strain of *Kitasatospora purpeofusca* that was repeatedly cultured in the presence of high selenite concentrations, highlight significant distinctions from its wild-type counterpart at the genome sequence level. Precisely, the genotypic variant of the *Kitasatospora* strain showcases attenuation of secondary metabolite production, placing paramount importance on selenite transformation. The attributes displayed by this *Kitasatospora* evolved strain position it as an asset in biotechnology.

B51 Beneficial symbiosis against desertification in Southern Europe: monitoring the mycorrhizal status of key plant species of semiarid agroecosystems

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Europe is increasingly affected by soil degradation processes that can ultimately lead to desertification. Vulnerability to desertification is most serious in European southern countries where soil erosion, salinisation, loss of soil organic carbon cause declining productivity with significant social, economic, and environmental consequences.

Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbiosis with more than 80% of land plant families with positive effects on plant health, resistance to abiotic stress, access to nutrients and C sequestration from the atmosphere. AMF are pivotal in the functioning of the ecosystems and they can significantly attenuate the effects of climate change. AMF spore density and root colonization are recognized as sensitive indicators of land degradation and restoration.

Within the project LIFE Desert Adapt (LIFE16CCA/IT/000011, www.desert-adapt.it/index.php/it/) we monitored, since 2018, the mycorrhizal status of twenty-one erbaceous and shrub species chosen as representative of different agro-ecosystems in nine sites of three European regions under critical desertification risk in Spain (Extremadura), Portugal (Alentejo) and Italy (Sicily). All the plants were found arbuscular mycorrhizal, including endemic species; some AM symbiosis were reported for the first time. High frequency of colonization (from 81 to 99%) was associated to variable intensity (from 29 to 72%) throughout all sites. Spore density (n. spores 100g soil $d.w^{-1}$) ranged from 9 to 144 with an evident decline observed in 2022 in respect to 2018. The AM data were processed to highlight correlations with soil chemical-physical parameters, soil biomass, land use and adaptation measures implemented in the sites within the LIFE Desert Adapt Project.

B52 Evaluation of the adhesion of selected hydrocarbonoclastic microbial strains to Biochar

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Bioaugmentation is a bioremediation strategy used to increase genetic diversity in polluted soil through the addition of specific microbial strains or consortium where a lack of microbial populations able to mineralize contaminants is observed. Its main limitation is to guarantee the establishment, the reproduction and persistence of the inoculated microorganisms which can be solved by immobilizing them on a carrier. As a matter of fact, biochar is raising interest among the scientific community and stakeholders as it represents a promising material for microbial immobilization due to its high carbon content, porosity, cation exchange capacity and abundant surface functional groups. Biochar is a stable porous carbonaceous solid produced through pyrolysis of biomass and it can be developed within the concept of circular economy, hence the production of this substrate allows the recycling of wastes allocating an added economical value to them. In this perspective, the aim of my PhD project is the activation of biochar with selected microbial strains to create a product to be applied in the bioremediation of petroleum hydrocarbon polluted soil. 24 microbial strains were selected and characterized for both their hydrocarbonoclastic abilities and plant-growth-promoting traits. Two among these strains were chosen based on the results of MATH assays to carry out adsorption test to understand how bacteria with different surface properties interact with biochar produced from different biomasses at different conditions of pyrolysis. This study will be a preliminary step for the optimization of the colonization of biochar from bacteria and for the production of a Microbe Activated Biochar.

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B53 In pursuit of a self-assembling protein decoy to counter Enterotoxic Escherichia coli F4+ strains causing piglet post-weaning diarrhea

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Post-weaning diarrhea (PWD) can cause severe losses in industrial pig farming. It is frequently caused by enterotoxigenic Escherichia coli (ETEC), expressing F4 or F18 fimbriae that bind to the intestinal microvilli of the piglets after weaning. Concerns about the emergence of antimicrobial resistance and environmental contamination have been raised globally due to the excessive use of antibiotics and zinc oxide to treat PWD. Therefore, alternative approaches to treat ETEC and preventing PWD are urgently needed.

This work explores the use of self-assembling nanoparticles as decoys to counter intestinal adhesion of ETEC $F4^+$ to microvilli. mi3, an icosahedral self-assembling protein, consisting of 60 monomers that form the mi3 scaffold, is bioengineered to display anti-ETEC F4 single domain antibody (sdAb, nanobody) fusions at the N-terminus of each monomer. The expression and purification of the bioengineered protein was optimized to obtain significant amounts of soluble

protein. The expression of the recombinant protein was optimized and purification of protein by Size Exclusion Chromatography (SEC) was validated by immunoblotting and Atomic Force Microscopy (AFM).
In vitro tests showed that the anti-ETEC F4-mi3 nanoparticles bind ETEC bacteria and trigger its accumulation, leading to their incapability of binding intestinal microvilli. These promising results were confirmed in independent microscopy assays and through western blot analysis and will be further tested and optimized. Currently, also other nanobodies recognizing different F4 fimbrial epitopes are tested using the same approach. In addition, methods to increase the yields of anti-ETEC F4-mi3 nanoparticles are investigated, including a SpyTag/SpyCatcher system for irreversible conjugation of recombinant anti-ETEC sdAbs to purify the mi3-SpyC scaffolds.

B54 Bioremediation of soils contaminated by weathered heavy oils: a case study

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Bioremediation of petroleum hydrocarbon (PH) contaminations can be considered as an established technology in the remediation market. Nevertheless, the biodegradation rate is largely dependent on the molecular structures. Moreover, there is a decrease in bioavailability, as the time of contact between contaminant and soil increases. Currently, the remediation of sites contaminated by heavy oils still entails landfill disposal in most cases, due to the lack of efficient and economically competitive alternative technologies. The investigated case study site is located in Sardinia (ex-SIPSA). There, petroleum refining first, later storage of heavy oils and bitumen, was carried out since the 1960s and reclamation started in 2014. Due to the contaminant properties, we considered the site an excellent test case for the development of novel bioremediation technologies of recalcitrant PH contaminations. In the framework of multidisciplinary collaborations between Accademia and private enterprises, we are testing and tuning biological methods for the process monitoring. Most probable number methodology was used for enumerating different metabolic groups of microorganisms able to degrade different PH fractions and the dehydrogenase activity was assayed for measuring microbial activities. Results will be presented of two technologies: i) an *in situ* biosparging test for groundwater treatment, ii) greenhouse tests of an integrated technology exploiting organic amendments and plants. Complementary to chemical analyses, biological parameters provided information on the microbial degradation, which are crucial for the decision-making process in remediation management. Currently, the characterization of bacterial and fungal communities by targeted metagenomic analysis of the rRNA genes is under evaluation.

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B55	***In vitro* screening and characterization of plant growth promoting rhizobacteria (PGPRs) isolated form rhizospheric soils of *Vitis vinifera* cv Falaghina: towards the development of a *terroir*-specific microbial *consortium***

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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 isolation of rhizospheric bacteria from the rhizosphere of *Vitis vinifera* L. cv Falanghina, vineyards in Guardia Sanframondi (BN), south of Italy, and the characterization for their direct and indirect plant growth promotion (PGP) capabilities. A total of 24 rhizobacteria were isolated and identified as *Bacillus* spp. and *Pseudomonas* spp. All the isolates were screened for *in vitro* ammonia, siderophores and indol-3-acetic acid (IAA) production, and for their antimicrobial activity against two important vine phytopathogens, *Rhizobium radiobacter* and *Botrytis cinerea*. In particular, *B. amyloliquefaciens* V6 isolate produced the highest ammonia amount, while *P. fluorescens* V2 and *P. aeruginosa* V2 isolates showed the best ability in siderophores producing. In addition, only *B. subtilis* V4 and *B. cereus* V4 isolates produced a significant amount of IAA. Finally, some isolates of *B. subtilis* (V4-V6) and *B. amyloliquefaciens* (V4-V6) distinguished for antibacterial and antifungal effects, respectively, against tested phytopathogens.

Obtained results showed that the isolates possess multiple PGP traits and can be considered as potential candidates for the formulation of a *terroir*-specific microbial consortium to be used along the wine chain as a valid eco-friendly strategy to promote sustainable agriculture.

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B56	**Bacteriophage-derived endolysins as novel strategy against Microbiologically Influenced Corrosion**

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Microbiologically Influenced Corrosion (MIC) consists in the deterioration of metal materials which is accelerated by microbial activities or their metabolites. Sulphate-Reducing Bacteria (SRB), such as *Desulfovibrio* spp., are the main responsible for MIC, by producing hydrogen sulfide, which is highly corrosive to iron and steel. The conventional use of chemical biocides to mitigate MIC generates detrimental effects on the environment, calling for the development of novel environmentally-friendly alternatives.

Recently, bacteriophage recombinant endolysins have been proposed as a novel class of innovative antibacterials, being effective in killing even multidrug-resistant strains. We intend to apply a similar approach to counteract *Desulfovibrio vulgaris* growth and, consequently, relieve

MIC. To this aim the Art-175 endolysin (Artilysin®), derived from a *Pseudomonas aeruginosa* phage and described as bactericidal against different Gram-negative bacteria, was used as model for the experimental design. Art-175 has been expressed, purified and tested *in vitro* against *P. aeruginosa* and *Acinetobacter baumannii*, to confirm its antibacterial activity. Interestingly, epifluorescence microscopy experiments have shown a promising killing activity of Art-175 even against *D. vulgaris* planktonic cultures, with a *ca.* 70% decrease in cell numbers compared to the untreated controls. Future work will be devoted on investigating the anti-biofilm properties of Art-175, against *D. vulgaris* biofilms, both grown in nutrient media and in artificial seawater, to better mimic the environmental conditions and the putative future applications. If successful, the use of more specific *D. vulgaris* bacteriophage endolysins could be regarded as a novel green approach to limit the SRB contribution to MIC.

B57 Microcosm biostimulation of anaerobic and aerobic dechlorinating bacteria in 1,2-dichloroethane contaminated water

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Chlorinated Aliphatic Hydrocarbons (CAHs) are persistent and toxic environmental pollutants. In contaminated groundwater, CAHs can be biodegraded by anaerobic Organohalide Respiring Bacteria (OHRB)[1] in the dehalorespiration process mediated by reductive dehalogenase enzymes. Nevertheless, aerobic oxidative processes can also co-occur, mediated by haloalkane dehalogenase enzymes, or cometabolically[2].

Specialized dechlorinating microorganisms can be stimulated by addition of proper amendments and exploited in bioremediation strategies. The aim of this work is to investigate the intrinsic biodegradation potential of a CAHs contaminated aquifer (mainly 1,2-dichloroethane, 1,2-DCA) by evaluating the response of the autochthonous microbial communities to biostimulation treatments in microcosm, under anaerobic and aerobic conditions.

Microcosms were set up from contaminated groundwater with appropriate mineral media under anaerobic conditions with (or without) lactate as electron donor to stimulate dehalorespiration, and under aerobic conditions with (or without) volatile hydrocarbon mixture to stimulate cometabolic or direct degradation. Parallel abiotic control microcosms were also set up. Chemical monitoring by Gas Chromatography-Mass Spectrometry over time revealed 1,2-DCA removal in all tested conditions. Illumina 16S rRNA gene sequencing revealed the enrichment of dechlorinating taxa in anaerobic microcosms, including known OHRB *Dehalococcoides* and *Desulfuromonas* and 1,2-DCA degraders such as *Ancylobacter* and *Starkeya*[3] in aerobic microcosms. The catabolic genes *rdhA* and *dhlA*, involved in reductive and hydrolytic dechlorination respectively, were detected in metagenomic DNA from microcosms by probing with specific primers. The biostimulation of anaerobic and aerobic 1,2-DCA degrading bacteria from the same groundwater suggests that both processes can be exploited for bioremediation purposes in site.

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B58 Biotic and abiotic influences on the ecological cycle of insect-associated yeasts

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Insects play a relevant role in the ecology and evolution of yeasts, in particular *Saccharomyces cerevisiae*, a well-known model microorganism with a still poorly defined ecological cycle. Social wasps vector *S. cerevisiae* all-year-long, pass it to other insects through vertical/horizontal transmission, and, over hibernation, promote the otherwise rare yeast mating. We still lack pivotal information on this multifaceted relationship. Primarily, we do not know whether the year-round detection of *S. cerevisiae* is due to a continuous exchange between adult wasps, rather than a long-lasting persistence in an individual, nor if the yeast permanence in active (not hibernating) wasps' gut would last enough to promote the yeast mating. To fill this gap, we monitored over time the presence of recognizable yeast cells administered to active *Polistes* wasps and the emergence of yeast mating products, hence gathering a detailed profile of the stability of the association. Another partially known aspect of the yeast-insect association is the range of yeast species that can be carried by social wasps and the role of the environment in defining the wasp mycobiota. To address this point, we censused the yeast populations vectored by social wasps in vineyards embedded in different environmental matrices. We found that in proximity to wooded areas, the vectored yeast populations show increased biodiversity and are characterized by the presence of oenologically relevant yeast species (e.g. *S. cerevisiae*). Overall, these results provide us with fundamental information to further understand biotic and abiotic impacts on the ecological cycle of insect-associated yeasts.

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B59 Application of bioelectrochemical systems for the remediation of a historically contaminated marine site in Chile

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Microbial electrochemical technologies may be a suitable strategy for the bioremediation of petroleum hydrocarbons-polluted sediments, by overcoming the electron acceptor limitation

because of the presence of an inexhaustible electron acceptor. In this work, BESs-based experiments were conducted to evaluate the bioremediation under anaerobic conditions of chronically petroleum hydrocarbons-polluted sediment of a brownfield in Viña del Mar (CHL). Three BES-based approaches were set up at a laboratory scale, including a microbial fuel cell, a microbial electrochemical cell, a microbial electrochemical snorkel (Closed Circuit, Pol-MERC, Snorkel) and ran in parallel with controls (Open Circuit, Natural Attenuation). These technologies were combined with the addition of compost to the sediment matrix. Even though the biodegradation of hydrocarbons was very low in the first 42 days of treatment, the sequencing of the 16S rRNA gene on metagenomic DNA showed a clear differentiation of the microbial community that was established on the anode from the ones on the cathode and in the sediment. On the anode of each system, there was an enrichment in genus of the Geobacteraceae family, known to be electrochemically active bacteria and hydrocarbon degrading bacteria. The Snorkel configuration showed the greatest enrichment, while the anode of the Open Circuit acted only as conductive support for the growth of these microorganisms. Chemical analyses suggest that probably sulphate reduction at the beginning of the experiment, and iron (III) reduction close to the anode after 42 days of treatment, are the main terminal electron-accepting processes in the systems. The systems stimulated the growth of anaerobic biofilm, starting point for the stimulation of anaerobic hydrocarbon degradation.

B60 Life CAPTURE, a Project for the Sustainable Management of Contaminated Sites by Per- and Polyfluoroalkyl Substances (PFAS)

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Poly and perfluoroalkyl substances (PFAS) are a group of synthetic organofluorinated compounds characterized by the presence of a fully or partially fluorinated carbon chain. These compounds have been used since the 1940s for a wide variety of industrial and commercial applications (e.g. s anti-stick coating on cookware, food packaging, cosmetics, waterproof textiles and firefighting foam).

The diffuse contamination by PFAS affected a large number of countries all over the world.

PFAS can enter the environment (soil and water) during the manufacturing process, usage of products and disposal of wastes, whereby the main contamination routes depend on the type of the application.

Unfortunately, due to the high C–F bonding strength PFAS are stable and highly persistent.

LIFE CAPTURE project intends to find innovative remediation solutions for PFAS-contaminated soil and groundwater in terms of sustainable management and treatment methods.

A first lab-scale trial has been started to test the possible inhibition of microbial growth by aqueous film-forming foam (AFFF) containing PFAS. A continuous bench scale activated sludge process has been setup, working at different concentrations of AFFF. Chemical Oxygen Demand (COD), pH, settleable solids and total suspended solids (TSS) are being monitored to select the optimal conditions for the growth of microorganisms and to assess possible inhibitory concentrations. In addition, the microbial community characterization is being performed by Next-generation sequencing and the quantification of cell abundance by qPCR. Finally, the ecotoxicity of AFFF, input and effluent of lab scale trial are testing (*Daphnia magna, Aliivibrio fischeri*).

B61 Space organic waste degradation by selected bacterial consortia: a new approach to microgreens cultivation in space

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Organic waste accumulated during human space missions represents a valuable resource in the perspective of future exploration programs of the solar system. Bringing enough food from Earth takes up volume and increases the spacecraft mass and fuel cost. A viable solution is the production of fresh food, such as edible microgreens in route using hydroponic systems and simultaneously recycling food and other organic space organic waste as fertilizer. Bioprocesses based on anaerobic digestion are commonly used on Earth for treating organic waste and were evaluated for adaptation to bioregenerative life support systems (BLSSs) in space contexts.

A mixture of organic food and non-food waste was prepared in laboratory, according to NASA data for missions aboard the International Space Station. Two bacterial consortia were selected directly from the space organic waste, through an enrichment process under anaerobic conditions. Metagenomic analysis revealed that the two consortia contain only eubacteria. In both consortia, most bacteria belong to the phylum *Firmicutes* with *Bacilli* representing the dominant class. As far as genera are concerned, the enrichment process caused a significant decrease of the relative abundance of *Lactobacillus* and a corresponding increase of the *Enterococcus* one. Upon degradation using these consortia, a significant mass reduction was observed and the resulting digestate proved to be suitable for microgreens cultivation. Indeed, phytotoxicity assays revealed that the liquid fraction of the digestate does not negatively affect microgreen seed germination, unlike untreated space organic waste. Moreover, *Raphanus sativus* was successfully cultivated as microgreen in hydroponic conditions using the liquid digestate.

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B62 Enhancing Microbial Production of Bioactive Metabolites through Mixotrophy and Salt Stress

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This project aims to improve the production of bioactive metabolites from novel isolates and model microbial strains by employing mixotrophy and salt stress. These metabolites hold potential as alternative solutions to combat antimicrobial resistance and reduce side effects on normal cells compared to conventional antibiotics and chemotherapeutic agents, respectively. To achieve this, we are investigating the potential of halophilic microorganisms from the saltern ponds of Trapani, an extreme and underexplored environmental niche characterized by varying salt concentrations and environmental conditions. By leveraging these natural biological and chemical variations, we

seek to harness the capabilities of halophilic microorganisms to produce bioactive metabolites. Model specie will be used as reference strains to compare with environmental isolates to produce these metabolites. Our study involved assessing the growth capacity of these strains under different salinity levels and trophic modes, including heterotrophy, mixotrophy, and phototrophy. The results revealed that salt stress enhanced cell repair activity on epithelial human cells and increased the concentration of molecules with interesting bioactivities in photosynthetic and heterotrophic new isolate microorganisms, respectively. Furthermore, employing mixotrophic conditions in the model microalga *Nannochloropsis granulata* demonstrated an increase in antitumoral activity against human prostate cell lines. Overall, our findings indicate that selected microorganisms can be cultivated in mixotrophic environments or under salt stress to produce high-value biomass containing antiproliferative and/or antioxidant compounds.

B63 Integrated molecular approaches for polyethylene degradation by *Rhodococcus opacus* R7 to investigate the degradative functions for biotechnological applications

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Plastic pollution is a global concern due to the high persistence of many synthetic plastics that have been accumulating in the environment. Their management has become a critical challenge, especially the highly recalcitrant polyethylene (PE) which accounts for around 60% of the total accumulated plastic waste.

A promising approach is the development of green technologies comprising effective microbial biodegradation for bioremediation and biotechnological applications.

Among the few PE-degrading bacteria, *Rhodococcus opacus* R7 can grow in the presence of untreated PE as the sole carbon and energy source in a short range of time. RNA-seq analysis performed on R7 cells grown on PE unveiled the complex genetic system of diverse oxidoreductases including three laccase-like multicopper oxidases (LMCO), an alkane monooxygenase, a cytochrome P450 hydroxylase, and membrane transporters.

In-depth investigations on the first step of oxidation through preliminary bioinformatic analyses and enzymatic assays on the supernatant of R7 grown in the presence of PE confirmed the activation of genes encoding LMCO enzymes. Two of them, LMCO2 and LMCO3, were selected and cloned for heterologous expression into an *E. coli-Rhodococcus* shuttle vector. Their production and characterization demonstrated that they belong to different LMCO families showing different biochemical and biophysical features.

The oxidative activity of R7 LMCOs on untreated PE assessed by FTIR and GC-MSD analyses up to 48 h revealed previously not described patterns of alkyl compounds and oxygenated products including ketones, alcohols, and carboxylic acids.

Structural analysis of LMCOs with density functional theory calculations shed light on LMCO mechanism of oxidation.

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 *Acinetobacter baumannii* OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence

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The outer membrane protein A (OmpA) plays a prominent role in biofilm formation, host cell infection, antibiotic resistance and immunomodulation in *Acinetobacter baumannii*. Combinations of OmpA and other outer membrane proteins (OMPs) enhance animal protection against *A. baumannii* infections. However, there are substantial gaps in our knowledge on *A. baumannii* OMPs. This study aimed to elucidate the role of OmpA-like proteins in *A. baumannii* strain AB5075 by means of isogenic mutants [ABUW_0505 (Δ*psaB*), ABUW_1015 (Δ*carO*), ABUW_2730 (Δ*arfA*), and ABUW_3045 (Δ*yiaD*)]. The wild type and *ompA* mutant were included as positive and negative controls, respectively. No differences in the antibiogram profile were detected. All mutants displayed a growth defect and lost twitching motility. Intriguingly, *ΔpsaB* showed an increased cell stiffness and permeability. Mutations in *psaB*, *arfA*, and *yiaD* loci led to a reduced cell envelope thickness. All mutants but Δ*psaB* were less adherent to lung epithelial cells. Δ*arfA* demonstrated a reduced biofilm-forming activity. A decreasing gradient of in vivo virulence was observed for all the mutants. The lack of PsaB, ArfA and YiaD significantly increased stress tolerance to human sera. However, the lack of PsaB results in increased tolerance to a number of different stresses. Our data indicate that PsaB has a crucial role in membrane fluidity that is critical in overcoming environmental stresses. ArfA contributes to the physiological cell envelope structure, aiding host cell adhesion. YiaD is involved in host-pathogen interactions. Hence, this study provides detailed clues about the role of under and not characterized yet OMPs in *A. baumannii*.

C14 The IN SIGNO project: Identification of novel molecules supporting the impact of β-lactams against clinically-relevant Gram-negative multidrug resistant organisms

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The spread of antimicrobial resistance represents an enormous global health crisis, with infections caused by multi-drug resistant bacteria contributing to >1 million deaths per year. A prompt action to limit the impact of this largely unmet medical need is mandatory, and it includes both the preservation of existing antibiotics and the identification of novel molecules. The aim of the IN SIGNO project, recently funded by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), is discovering -through a biological activity-guided screening- novel antibiotic adjuvants, which could be used in combination with β-lactams against multidrug-resistant Gram-negative (MDR-GN) bacteria. To this purpose, we first selected five clinically-relevant models of β-lactam resistance in MDR-GN: an extended-spectrum β-lactamase-producing *Escherichia coli* strain (CTX-M type), three carbapenemase-producing *Klebsiella pneumoniae* strains (KPC-1, KPC-31, and VIM-1 types), and a VIM-2-producing *Pseudomonas aeruginosa*. Then, we proceed with the screening of a filamentous actinomycetes/fungi-based microbial library (39,000 crude extracts) and of a chemical library (9,500 pure compounds), to select molecules able to restore the activity of β-lactams against the resistant isolates previously described. The three most promising candidates thus far selected are natural products, since no hits have been identified yet from chemical library. If their activity is confirmed, we will progress in elucidating the chemical structure of the putative inhibitors, test their *in vitro* and *in silico* interaction with β-lactamases to better define their mode of action, evaluate activity on a wider panel of MDR-GN clinical isolates, and assess their cytotoxicity on different eukaryotic cells.

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C15 Characterisation of a novel phage species of *Staphylococcus aureus* belonging to Kayvirus

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Antibiotic resistance represents a challenging global threat. The development of new antimicrobial therapies for the control of infectious diseases is needed. Bacteriophages represent a natural source of antibacterials. The aim of this work was to isolate and characterised a novel *Staphylococcus aureus* phage for therapeutic use.

Phage isolation was carried out by enrichment procedure inoculating *S. aureus* ATCC43300 in the

presence of wastewater samples of different origin. Phage genome sequencing was performed by Illumina and Blast tools. Phage were phenotypically characterized by performing a one-step growth curve and testing their stability after 1-hour exposure to different pH and temperatures. A phage killing kinetics was also carried out for24-hours to evaluate the selection of phage-resistant clones.

The genotypic characterization identified Zeno as a new *S. aureus* virulent phage species belonging to the Kayvirus genus and presenting a 150 Kb genome with no antibiotic resistance genes, toxin, or virulence factors. Phage Zeno has a latent period of 20 minutes with a burst size of 10^5 PFU/mL, it remains stable at pH ranging from 4 to 11 and also at temperatures between 37°C and 50°C. The lysis kinetic curves showed that four out of five bacterial clones, selected from colonies growth after 24h phage-bacteria, exhibited phage resistance evolved during the first treatment with Zeno. Resistant clone sequencing/analysis is still on-going.

Due to its genotypic and phenotypic features, Zeno is a promising candidate the treatment of drug-resistant *S. aureus*-associated infections.

C16 A picture of the vaginal microbiome during pregnancy and puerperium

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Background:
The composition of the vaginal microbiome can vary throughout a woman's life and, it undergoes marked changes during pregnancy. The aim of this study was to analyze the vaginal environment and identify the presence of antibiotic resistance genes in a cohort of women throughout different gestational ages and puerperium.

Methods and Results:
For each subject (n=63) and time point (n=4), both the vaginal bacterial composition (16S rRNA sequencing) and the vaginal metabolic profiles (1H-NMR) were analyzed.
During the pregnancy evolution, we found a significant increase of Lactobacillus and related metabolites (lactate and amino acids) and a decrease of bacterial vaginosis (BV)-related bacteria, whereas, during the puerperium, we observed the opposite behavior. BV and the presence of Megasphaera were correlated to the positivity to antibiotic resistance genes (ermB, ermF, tet(W)). Finally, Gardnerella vaginalis clade 2, or the presence of multiple clades, was related to major shifts in the vaginal microbial composition.

Conclusions:
A deep comprehension of the vaginal ecosystem may unravel pregnancy's pathophysiology and provide markers to identify women at risk of complications. Considering that microbial communities can be transferred from the mother to the newborn, this study can open new perspectives for infant's microbiome development and future health.

C17 SOGA1 activity against *Porphyromonas gingivalis* and analysis of Outer Membrane Vesicles (OMV) production

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Porphyromonas gingivalis is an anaerobic, Gram-negative, rod-shaped bacterium. It is a pathogen active in the most severe forms of periodontal disease and possibly involved in neurodegenerative disorders such as Alzheimer. Its virulence factors include LPS, fimbriae, capsule and gingipain, a group of cysteine-proteinases of the outer membrane and released by outer membrane vesicles (OMV) by the bacterium itself. Given the small size of OMVs (50-250nm) these cross the bloodstream and reach more easily the blood-brain barrier (BBB), promoting increased levels of inflammatory cytokines, cellular and vascular adhesion molecules. In this study we evaluated the ability of OMVs to promote the aggregation of bacterial cells and to favour biofilm formation. In order to find new molecules active against *P. gingivalis* and its OMVs, we screened a group of human derived peptides. The most promising was SOGA1, which was active against *P. gingivalis* and other potential oral pathogens. This peptide is hydrophobic and positively charged, targets the bacterial membranes and could be involved in the mechanism of OMV production.

C18 Two years of COVID-19 vaccination: longitudinal analysis of the spike-specific immune response

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The mRNA vaccines have been used for the first time in the context of the SARS-CoV-2 pandemic, demonstrating efficacy and immunogenicity in the real-world setting. During these two years, we have longitudinally assessed the spike-specific antibody and memory B cell responses, in a cohort of 227 healthy subjects enrolled in the IMMUNO_COV study. Subjects have been stratified into different groups according to the vaccination schedules received, and the SARS-CoV-2 infection, to characterize the persistence of the immune response elicited by different vaccine schedules, and the impact of the natural infection (hybrid immunity). Spike-specific IgG were analysed by ELISA and a surrogate of neutralization assay, while B cells were identified by flow cytometry with computational analysis of data.

Significant levels of spike-specific IgG were induced since the second vaccine dose. A stable persistence of humoral response was detected up to 9 months, after a physiological decline observed upon three months. Humoral response was boosted by the third vaccine dose, eliciting antibodies capable of binding the Delta and the Omicron RBD variants. Spike-specific memory B cells were generated by mRNA vaccines and persisted 6 months after booster dose. The impact of hybrid immunity and the immunological analysis performed at month 24 are ongoing.

The present research significantly contributes to gain insights into the immunogenicity of SARS-CoV-2 vaccination and long-term persistence of the immune response, and it can help in guiding future decisions and vaccination schedules.

C19 *Mycobacterium abscessus* siderophores biosynthesis as target to inhibit the iron uptake mechanism

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Mycobacterium abscessus (Mab) is a rapid-growing non-tuberculous mycobacterium, which is emerging as an opportunistic pathogen in patients with lung disorders, such as those with cystic fibrosis. With the aim of searching for new anti-virulence treatments, which do not kill the bacteria but disarm them before attacking the host, we focused on the siderophores biosynthesis. Mab produces siderophores to scavenge iron from the host, that are essential for the establishment and maintenance of infection. The salicylate synthase (Mab-SaS), the first enzyme involved in the biosynthesis of siderophores, catalyses the conversion of chorismate into salicylic acid. Its homologue in *M. tuberculosis* (Mtb-MbtI) has already been extensively characterised and identified as a potential therapeutic target, and promising phenylfuran-carboxylate based inhibitors were developed. Taking into consideration the successful work performed on *M. tuberculosis*, the aim of this work is therefore to identify compounds capable of inhibiting the enzymatic activity of the Mab-SaS, starting from the library of compounds developed for Mtb-MbtI. The Mab-SaS was then produced in recombinant form, and its enzymatic activity established by a fluorimetric assay. Upon screening of the compounds belonging to the library of Mtb-MbtI inhibitors, some showed good activity even against Mab-SaS, with IC_{50} values in the low micromolar range, and not behaving as PAINs. These results support the hypothesis that phenylfuran-carboxylate is a promising scaffold for the inhibition of Mab-SaS as well, paving the way for the optimization and rational design of more potent derivatives.

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C20 Molecular engineering of a nanotheranostic phage-based platform: a versatile modular approach for targeted therapeutics and biosensing applications

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Phage-based targeting platforms have emerged as versatile tools in the field of biomedical research and therapy. Here, we present the development of a novel targeting platform utilizing the M13 phage, genetically engineered to display various targeting moieties (peptides, nanobodies, or scFv) in fusion with the pIII protein on the phage tip, allowing for precise molecular recognition and binding to specific targets. Additionally, we successfully chemically conjugated photosensitizers or fluorescent dyes onto pVIII proteins, resulting in a nanovector capable of delivering hundreds of molecules precisely to their intended target. This innovative strategy has enabled the development of a modular and multifunctional platform for theranostic and biosensoristic applications.

As proof of concept of the modularity and potentiality of the platform, it was demonstrated the selective targeting of engineered phage to cancer cells (HER2 positive cells), bacterial cells (*A. baumannii*) or viruses (SARS-CoV2). Next, bioconjugation of phage with Rose Bengal (RB) allows to transform these targeting agents in photoactivable vectors, which precisely targets and eradicates either cancer or bacterial cell. Remarkably, phage-based platform demonstrated antimicrobial and antitumoral photodynamic therapy (PDT) efficacy at picomolar vector concentrations. Moreover, the versatility of the developed platform extended to the field of biosensing. By incorporating the specific targeting moieties, the engineered M13 phage was able to detect and bind to SARS-CoV-2 virions, enabling the creation of a sensitive biosensor for the rapid detection of the virus.

The development of such versatile phage-based systems opens new avenues for personalized medicine and disease detection in the field of biomedicine.

C21	**Design of a new *in vitro* reconstructed human gut microbiota: a perspective on its modulation by prebiotic molecules and probiotics leading to benefits for human health**

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This study aims to design and characterize a new reconstructed *in vitro* human gut microbiota and to evaluate its modulation by the presence of new prebiotics and probiotics inducing health-promoting effects on human cells.

The *in vitro* model comprised representative bacteria of the real gut microbiota (Firmicutes, Bacteroidetes, and Proteobacteria), which was challenged with probiotics (Lactobacilli and Bifidobacteria) in the presence of prebiotic Maitake extract.

Preliminarily, the *in vitro* model was validated using known prebiotic molecules (3-5 DP FOS) monitoring both the growth levels of the strains and the production of metabolites.

Then, the modulation of the *in vitro* reconstructed gut microbiota was evaluated in the presence of the Maitake extract (glucans-rich) through the measurement of the growth and by species-specific qPCR analyses. The obtained results demonstrated that the Maitake extract principally sustained the growth of the probiotic bacteria. In particular, the growth of *Lactiplantibacillus plantarum* and *Bifidobacterium animalis* subsp. *lactis* carried out the production of secondary metabolites, such as SCFAs and BCFAs, and hydrocinnamic acid. Finally, the exposure of human epithelial (HT-29) and immune cells (PBMCs) to the Maitake-fermentated metabolites resulted in beneficial effects, in terms of protection against ROS (increasing SOD1 and NQO1 levels in the epithelium) and induction of immunoregulatory IL-10, skewing all the immunological compartment to a tolerogenic microenvironment.

Therefore, the prebiotic Maitake extract and the selected probiotics were effective in the modulation of the *in vitro* model of human gut microbiota, whose metabolites exerted positive health-promoting effects on epithelial and immune cells.

C22 Yet another potential microbial player in autoimmunity: multicentric analysis of the association between *Mycoplasma hominis* serostatus and rheumatoid arthritis (RA)

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RA is a systemic inflammatory disease of autoimmune origin, in which genetic background is of paramount importance. Genetic factors alone do not fully explain RA pathogenesis, and the contribution of environmental factors including mucosal infections has gained growing attention. Among mucosal pathogens, *M. hominis* typically cause chronic infections, allowing persistence in the host over extended periods of time, suggesting an ability to modulate host immunity.

Since *M. hominis* is a potent inducer of Neutrophil Extracellular Traps, an innate immune mechanism associated with rheumatoid arthritis (RA) onset and progression, we designed a serological multicentric study aimed at assessing the prevalence of anti-*M. hominis* antibodies (Abs) in two independent groups of RA patients.

Anti-*M. hominis* antibodies were detected in RA patients' sera collected from 2 independent centres (Sassari, Italy; Hue, Vietnam) as compared to matched control populations, with significant differences in both optical densities and Ab detection between the two groups (overall 75% vs 41%; p value <0,0001). The specificity of the immune reaction was shown by means of western blot experiments, showing the binding of RA sera-derived Abs to *M. hominis* antigens. No immune reactivity was detected in ELISA-negative sera. Interestingly, we couldn't observe any significant differences in *M. hominis* serostatus between italian and vietnamese groups. These data may suggest a hypothetical role of *M. hominis* as a trigger in RA pathogenesis. Whether RA and *M. hominis* serostatus are actually linked by a cause-effect relationship need more research aimed at elucidating the potential contribution of *M. hominis* to RA.

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C23 Genotypic and phenotypic characterization of new species of mycobacteriophages active against *Mycobacterium abscessus*

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The bacteriophage use has re-emerged as a promising therapeutic option for the treatment of drug-resistant bacteria, including *Mycobacterium abscessus*. This study aimed to isolate and characterised novel mycobacteriophages.

Forty-two environmental samples were inoculated in broth with *M. smegmatis* mc^2 155 as host. After enrichment procedure, phages were isolated and their genomes were extracted, sequenced (by Illumina) and annotated. The phages were characterized by one-step growth curves. Then, their lytic activity against *M. abscessus* clinical strains was tested by spot assay.

Three different phages were isolated from two soil samples and propagated in *M. smegmatis* mc^2 155. Whole genome sequencing and annotation reviled that the mycobacteriophages were temperate and belonged to two novel species of genus *Anayavirus* and *Benedictvirus*. The length of the genomes ranged from 50 and 60 Kbp with 60-67% of CG content. Integrase gene was found in all phage genomes, while repressor gene was present only in two out of three phages. No genes encoding for drug resistance, toxins or virulence traits were identified. Their one-step growth curves showed one phage was able to fast lysate cells (within 20 min). Finally, phages were able to infect and kill all tested *M. abscessus* strains, including isolates associated with disseminated infection and multidrug resistance.

New species of mycobacteriophages were identified and characterized. The absence of drug resistance, toxins or virulence genes, the fast replication cycle and the lytic activity against *M. abscessus* clinical strains, make the three phages potentially useful for the development of phage-based treatment of *M. abscessus*.

C24 Acetylation of H3K56 in *Candida albicans* affects the J744.1 macrophage response

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Candida albicans is an opportunistic fungus of the human microbiota. Alterations in host-microbiota balance can induce overgrowth of this species causing a wide range of infections, from superficial mucosal to disseminated candidiasis.

Tissue-resident macrophages function as the first line of defence against fungal infections and trigger host immune responses. *C. albicans* has evolved several strategies to escape macrophage engulfment and successfully establish systemic infection. Although the critical role of chromatin structure on gene expression is undeniable, the contribution of epigenetic mechanisms underlying the expression of virulence genes in *Candida* is largely unexplored. The most abundant epigenetic modification in *C. albicans* is the H3K56ac, resulting from the activity of two opposite enzymes: the histone acetyltransferase Rtt109p and the NAD^+-dependent histone deacetylase Hst3p.

We aimed to elucidate the role of such chromatin modification by looking at the phenotype and gene dysregulation produced by nicotinamide, a sirtuin inhibitor. Our data show that Hst3p inhibition promotes the accumulation of H3K56 acetylation, which leads to a defective hyphal elongation, one of *Candida* main virulence strategies. In these conditions, *C. albicans* secretes soluble factors which promote phagocytosis in J774A.1 murine macrophages.

To determine which genes are involved in the dysregulation of H3K56ac we performed a ChIP-seq analysis with anti-H3K56ac antibody and an RNA-seq analysis on *C. albicans* treated with the sirtuin inhibitor *versus* the control.

The genome-wide analyses confirmed that this epigenetic modification contributes to triggering the host's susceptibility towards the pathogen, for instance, by modeling its cell wall and preventing recognition and, therefore, phagocytosis.

C25 Indolyldiketoacids inhibit SARS-COV-2 nsp13 and viral replication exhibiting broad spectrum antiviral activity

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The recent SARS-CoV-2 pandemic highlighted the risk of a zoonotic spillover into human population, bringing the attention on the need of the development of pan-coronaviruses antivirals. For RNA viruses, RNA helicases have long been recognized to play critical roles during virus replication cycles, facilitating proper folding and replication of viral RNAs, therefore representing an ideal target for drug discovery.

SARS-CoV-2 helicase, nsp13, unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. Nsp13 is a multidomain enzyme that couples two C-terminal RecA ATPase domains, characteristic of the 1B (SF1B) helicase superfamily, with other three domains: the N-terminal zinc-binding domain (ZBD), essential for the helicase activity, a stalk, and a 1B domain. Nsp13 is a highly conserved protein among all known coronaviruses, and, at the moment, is one of the most explored viral targets to identify new possible antiviral agents.

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In the present study, we present indolyldiketoacid (DKA) derivatives, as nsp13 inhibitors, investigated coupling molecular biology, in silico modelling and cell-based assays.

Among them, four compounds inhibit viral replication in the low micromolar range and block both nsp13 enzymatic functions. Mode-of-action studies revealed ATP-non-competitive kinetics of inhibition, not affected by substrate-displacement effect, suggesting an allosteric binding, into an allosteric conserved site located in the RecA2 domain, molecular modelling of binding pose predict interactions with conserved residues within the putative binding site. DKAs showed the capacity to inhibit viral replication of other HCoVs exhibiting a broad-spectrum antiviral activity providing strong rationale to exploit DKA's scaffold to identify new HCoVs inhibitors.

C26 Evaluation of staphylococcal phage lytic activity in human serum

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Systemic phage administration represents the most suitable strategy to treat bacteraemia. In this end, we evaluated the *in vitro* lytic activity of Sb-1 phage against methicillin-resistant *Staphylococcus aureus* (MRSA), in the presence of either whole serum or its components.

The activity of Sb-1 was assessed against MRSA by pre-incubating/co-incubating both bacteria and phages in the presence of 30% (v/v): i) heat-inactivated sera from different donors, including

human, bovine and fetal calf (FCS) samples; ii) heat-inactivated serum (80°C for 30'); iii) serum depleted of IgG (PANSORBIN); iv) Bovine Serum Albumin (BSA). The lytic activity of Sb-1 was also tested on bacteria pre-incubated with human serum and subsequently washed with Triton X-100 0.1% (v/v).

Bacterial eradication was observed when cells were incubated for 24 h with 10^8 PFU/mL phages, but not in presence of either human or bovine serum, suggesting an inhibitory effect of serum on phage activity. When Sb-1 was tested against MRSA in the presence of diluted human serum up to 1:256 and PANSORBIN-pre-treated, no CFU reduction was observed. The incubation of Sb-1 with serum did not reduce the phage titer, suggesting that no phage neutralization occurred. In the presence of either FCS or BSA, Sb-1 exerted bactericidal activity on MRSA. Notably, when MRSA cells were pre-incubated with human serum and then washed with Triton X-100, Sb-1 caused bacterial eradication. The lower amount of lipids in FCS and the observed recovery in phage lytic activity after Triton X-100 treatment suggest that human serum lipids might affect the Sb-1 lytic activity.

C27 Dynamics of macrophage phagocytosis

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Streptococcus pneumoniae is one of the major causes of serious disease and death and about half of patients with pneumonia have sepsis. We and others have focused for many years on the mechanism by which pneumococci invade cells, including professional phagocytes which seems to be the starting point for septicaemia.

To describe pneumococcal intracellular survival, we performed invasion/adhesion and killing assays and time-lapse confocal microscopy of unencapsulated GFP tagged pneumococci, fluorescent beads and J774.1 macrophages at a MOI of 10. Image analysis was performed to score the invasion events on films spanning 20 to 30 minutes and visualising 10 to 20 macrophages.

The analysis shows that over 80% of macrophages took up multiple pneumococci with an average of 13 bacteria per macrophage with a SD of 14 and a cell lysis of about 20%. The average score of the phagocytosis events was 140 with a SD of 60. In a well seeded with 10^5 macrophages this would equivale to uptake of almost all pneumococci. When analysing the approximate 50 to 500 pneumococcal invasion events in a normal cell-invasion assay, a complete killing of bacteria was observed except for about hundreds. We had focussed on analysing factors allowing pneumococcal survival in cells.

Our current data indicate that the uptake events appear to be about 10E6 in the same time frame. We might therefore have to re-focus our question in asking what happens in the very rare cells (1 in 10E-4) where efficient killing is blocked leaving alive pneumococci within a phagocyte.

C28 Development of a model to study the interaction of *Pseudomonas aeruginosa* with airway epithelial cells

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Pseudomonas aeruginosa is classically defined as an extracellular opportunistic human pathogen, able to form antibiotic-resistant multicellular communities called biofilms. However, several studies have shown that *P. aeruginosa* is internalized and survives inside not specialized phagocytic cells. Although intracellular survival of *P. aeruginosa* could be an important strategy to evade the host immune system and escape antibiotic activity, it remains poorly characterized.

We developed an *in vitro* model to investigate the *P. aeruginosa* intracellular lifestyle and the main bacterial pathways required for its survival within human airway epithelial cells.

A549 lung carcinoma epithelial cells were infected with the *P. aeruginosa* PAO1 laboratory strain constitutively expressing the *gfp* reporter gene. Following the initial internalization phase, extracellular bacteria were killed with amikacin/gentamicin. Bacterial association with infected cells was studied for up to 24 hours. Bacterial survival was monitored using the CFU count method and, in parallel, analyses at single-cell level were conducted using confocal microscopy and flow cytometry. Different cultural conditions and different *gfp* expression systems were tested.

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Our results showed that *P. aeruginosa* survives within A549 for up to 24 hours after infection. Experiments are underway to study the impact of the main *P. aeruginosa* regulatory systems (*e.g.*, quorum sensing, stringent response) on bacterial intracellular survival and on the response of A549 to bacterial invasion. Preliminary results suggest that the stringent response plays an important role in the intracellular lifestyle of *P. aeruginosa*.

C29 Deciphering the interactions between macrophages and bacteria in *ex vivo* human spleen and liver perfusion models

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Tissue resident macrophages are a heterogeneous population that provides innate cellular immunity by performing pathogens removal from the bloodstream and protects from sepsis during systemic infections. Previous studies in mouse models showed that invasive bacterial disease occurs after rare events of macrophage functionality failure that led to within-macrophage replication of bacteria and consequent systemic infection. However, the mechanisms of interaction between macrophages and bacteria in the human spleen and liver are still poorly known, notably in the early phases of the infection process. To this end, we utilized *ex vivo* human spleen and liver perfusion models to investigate the interactions between macrophages and a cocktail of *Streptococcus pneumoniae* serotypes with high or low invasive disease potential. Spleens and

livers were collected, infected, and perfused for 6 hours. Biopsies were collected at different timepoints, processed for viable bacterial cell count, and stained for immunofluorescence for macrophages subtypes, cell apoptosis, and bacterial markers. Microscopy of spleen biopsies revealed that most bacteria were detected inside the red pulp macrophages. Apoptosis was observed about eight times more frequently in perifollicular macrophages that are among the first cell types to encounter invading pathogens. Differently to the spleen, the liver macrophages were less efficient in pneumococcal clearance and no significant differences in the uptake of low and high virulent serotypes was observed. These first perfusion models indicate the different efficiency of spleen and liver macrophages in the clearance and the presence of a relationship between apoptosis events and bacterial removal.

C30 Nanobiotechnological engineering of the M13 phage for targeted photodynamic Ovarian cancer therapy

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Bacteriophages, the viruses of bacteria, are of great importance for future nanomedicines. They play a crucial role not only in the detection of bacterial infections and as alternative antibiotics but can also be used as nanobiotechnological platforms to target malignant cells and deliver drugs specifically where needed. Ovarian cancer is the most lethal gynaecologic malignancy and patients suffer particularly often from cancer recurrence after treatment. An effective and targeted therapy is therefore urgently needed to combat Ovarian cancer efficiently.

This work presents a novel photodynamic Ovarian cancer therapy based on the bacteriophage M13 as a nanobiotechnological platform that targets cancer cells specifically.

The pIII protein, expressed on the tip of the M13 phage, was genetically modified to display a nanobody or single chain fragment specific for either EGFR (epithermal growth factor receptor) or FRα (Folate receptor alpha). These receptors are overexpressed on different Ovarian cancer cell lines and thus present a reliable cancer marker and target. The pVIII protein of the recombinant M13 phages is conjugated to Rose bengal, a photosensitizer that produce ROS (reactive oxygen species) upon light activation. ROS leads to the death of the cells.

The recombinant phages were shown to target Ovarian cancer cells specifically, also after conjugation with the photosensitizer. Furthermore, it was demonstrated in *in-vitro* assays that bioconjugated phages effectively deplete cancer cells in a dose dependent manner and only upon light irradiation. These results indicate a promising novel cancer therapy that must be further developed and tested.

C31 *Pseudomonas aeruginosa* viable but non-culturable forms in cystic fibrosis patients: role of antibiotic- and modulator-based treatment in the pathogen survival and adaptation

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Pseudomonas aeruginosa (PA) Viable But Non-Culturable (VBNC) cells contribute to the failure of pulmonary infection eradication in Cystic Fibrosis (CF) patients. Antibiotic-exposure could favor the induction and persistence of these dormant bacterial forms.

In this study (Project Grant FFC#22/2020), 94 CF patients afferent to the Ancona CF center were monitored for 12 months for the presence of PA VBNC cells in the sputum and a correlation between their detection/abundance and antibiotic/CFTR modulator-therapies was investigated. VBNC abundance was calculated as difference between qPCR (performed by a previously-validated *ecfX*-targeting assay) and CFU counts. Only differences >0.5log were considered as indicative of the VBNC cell presence.

PA was detected in 52 of 79 patients with documented chronic lung infection.

Among these, 46 (88.5%) presented VBNC forms. In three patients the infection was temporally cleared for 2-4 months and in four for 3-6 months until the end of the study, all were treated with the CFTR modulator elexacaftor/tezacaftor/ivacaftor (ETI), the latters also with antibiotics.

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Eight patients shifted from culture-negative/qPCR-positive to culture-positive samples, likely due to VBNC cell reactivation in absence of antibiotic therapy.

Finally, 12/24 patients treated with aminoglycosides for a PA pulmonary exacerbation, exhibited a persistent VBNC subpopulation after the end of therapy, in agreement with our previous *in vitro* data.

These results strongly support the persistence of PA VBNC cells in the CF lungs upon antibiotic/ETI treatment; the study of their long-term efficacy and the design of novel strategies to counteract the pathogen adaptation to the CF lung are therefore warranted.

C32 Integrated strategies to overcome the lack of novel antibiotics against Gram-positive pathogens

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Antimicrobial resistance (AMR) represents an enormous global health crisis. However, novel antimicrobial drugs to tackle AMR spread are increasingly difficult to discover and develop, thus creating a widening gap between clinical needs and drug pipeline innovation[1].

In this context, our research efforts in the recent years have been two-pronged. On the one hand, we investigated the use of nanotechnology-tailored agents as alternative antibacterial treatments that could potentiate the efficacy of already-in-use antibiotics. We successfully prepared nano-formulations of the 'last-resort' glycopeptide antibiotics (GPA) teicoplanin and vancomycin -clinically used for fighting severe infections caused by multi-resistant Gram-positive

pathogens- by conjugating them to iron oxide nanoparticles[2,3]. In addition to presenting good stability, reduced cytotoxicity, and *in vitro* antimicrobial activity against a panel of Gram-positive pathogens, when concentrated by the action of an external magnet, these superparamagnetic nano-antibiotics exerted a localized inhibition on *Staphylococcus aureus* biofilm formation even at low GPA concentration, at which the effect of the free counterpart was negligible[3].
On the other hand, to limit the use of mammals in drug discovery and development -which determines serious ethical problems, high expenses, and long times, thus slowing down preclinical tests of new drugs-, we developed an alternative infection model based on the silkworm *Bombyx mori*, demonstrating its usefulness for evaluating GPA candidates against staphylococcal infections[4,5]. Recently, the infection model proved its usefulness also for acquiring data on *in vivo* toxicity and efficacy of the previously prepared nano-antibiotics, thus extending its potential future applications.

[1]Berini et al. 2022. doi: 10.1016/j.biotechadv.2022.107948
[2]Armenia et al. 2018. doi: 10.3389/fmicb.2018.02270
[3]Berini et al. 2021. doi: 10.3389/fmicb.2021.657431
[4]Montali et al. 2020. doi: 10.3390/antibiotics9060300
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C33 Identification of microRNAs possibly involved in the cellular innate response to herpes simplex virus 1 infection

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MicroRNAs (miRNAs) are noncoding RNAs that finely regulate gene expression by blocking translation or promoting degradation of target messenger RNAs. Based on ligand/receptor interactions, a wide range of cell types can be infected by herpes simplex viruses 1 (HSV-1). However, a full replication cycle can be, normally, completed by HSV-1 only in epithelial or neural cells. Complex mechanisms controlling the innate response to HSV-1 infection in a cell-dependent manner, have not been fully elucidated. Preliminarily, cellular miRNAs with high score of predictability to be involved in the innate response to HSV-1 were selected by bioinformatics software. One tool utilized information from databases to predict the interaction of cellular miRNAs with HSV-1 transcription products, while another one matched data from a miRNAs array performed in U937 cell lines with functional or non-functional NF-kB activation and data from databases on miRNA targeting innate-response-related genes. Based on the arbitrary scores obtained from bioinformatics miR-762 and miR-99a-5p were selected. Successively, these data were validated by evaluating, using qRT-PCR, the expressions levels of miRNAs in cells infected with HSV-1 under known, different conditions of innate response to infection. Results showed a significant correlation between expression of miR-762 following HSV-1 infection, with respect to mock-infected cells, and the innate response to infection of different cell types, and that miR-99a-5p expression was quite equal in HSV-1-infected U937 cells with functional NF-kB activation, i.e. an effective innate response, and mock-infected cells, while it was highly expressed with respect to mock-infected in HSV-1-infected U937 cells with non-functional NF-kB activation.

C34 *Galleria mellonella* and Zebrafish as suitable infection models to study nutritional immunity mechanisms

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To limit the growth of pathogens, vertebrates evolved innate immunity defence mechanisms described as "nutritional immunity" based on the modulation of the concentration of metals such as zinc in the environment. *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen able to grow despite limited supply of zinc due to efficient metal-acquisition systems. Numerous studies conducted in mammalian models aimed at examining the interaction between PA and its host. However ethical and financial issues related to these models increased the interest in exploring alternative organisms. *Galleria mellonella* (GM) and Zebrafish offer several advantages including shorter generation times and lower maintenance costs. Moreover, their immune system exhibits remarkable similarities to that of mammals, making them suitable for studying host-pathogen interactions. To investigate whether GM larvae and Zebrafish embryo possess nutritional immunity mechanisms comparable to mammals, we infected these organisms with PA wild-type and mutant strains with reduced zinc-import ability. We observed that 18 hours-post-infection of GM larvae, strains lacking the main zinc-importers significantly lose their colonization ability compared to the wild-type, and this was also evident 48 hours-post-infection of Zebrafish embryos, suggesting that PA faces zinc-depleted environments while colonizing both organisms. However, expression analysis of the gene encoding a zinc-efflux pump revealed that at early infection stage of Zebrafish embryos, PA is exposed to high zinc-concentrations, suggesting an antimicrobial mechanism based on zinc-intoxication. These results provide evidence that both organisms share similar nutritional immunity mechanisms with higher eukayiotes, supporting their usefulness as animal models to study the interaction between PA and its host.

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C35 Phage-resistance in *Klebsiella pneumoniae* clinical isolates correlates to augmented clearance by phagocytes

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Hospital-acquired infections sustained by *Klebsiella pneumoniae* (KP) strains are often very difficult to treat, mainly due to their frequent association with complex antibiotic resistance traits. Recently, the therapeutic use of bacteriophages against multidrug resistant bacterial infections is gaining a renewed interest, although its efficacy can be limited by the insurgence of phage-resistant strains, possibly making this approach inefficient and requiring constant efforts for new phage isolations. Since we have previously reported that a φBO1E resistant *K. pneumoniae* strain (BO-FR-1) shows a significant reduction of virulence in a *Galleria mellonella* model, in the present study we further evaluate the effect of phage resistance on host-pathogen interaction.

Two KP phage-resistant mutants, BO-FR-1 and KP263-FR, have been generated *in vitro* using the KKBO-1 (Sequence Type 258) or KP263 (Sequence Type 147) clinical isolates. Bacterial

clones were then used to infect monocyte-derived type-1 and type-2 macrophages obtained from healthy donors. Macrophage response was finally evaluated in terms of internalization index, intracellular killing capability, and inflammatory response.

Our results show that both phage-resistant clones were significantly more prone to phagocytosis and to intracellular killing when compared to their parental strains, without showing significant differences in NF-kB activation.

Although the insurgence of phage-resistance may represent a limitation for phage therapy, in the context of the interplay among phage, bacterial pathogen and host, it may be beneficial for the host by making bacterial strains more susceptible to innate immune response.

C36 Transcriptomic profile of murine macrophages in response to *Brucella abortus* infection

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Brucella is a facultative intracellular bacterium causing brucellosis, an important zoonosis worldwide. *Brucella* virulence depends on its ability to replicate in host cells and in particular, the macrophage-*Brucella* interaction is critical for establishing chronic infections; immediately after invasion, *Brucella* resides in the brucellosome and begins to multiply. Additionally, to promote its persistence, *Brucella* inhibits macrophage apoptosis, which in turn favors pathogen survival and replication. However, the molecular mechanism of this phenomenon is still unclear.

To elucidate the biological changes occurring in host cells in response to *Brucella* infection and their potential impact on the final outcome, we examined the transcriptional profile of murine macrophages J774.1 infected with *B. abortus* isolate from local Buffaloes. RNA samples were collected at 2, 6, and 24 hours after infection. After 2 hours a total of 1067 genes were differentially expressed, while after 6 and 24 hours of infection, 4299 and 7600 genes showed differential expression, respectively. Throughout the infection, we observed the activation of several signaling pathways, including cytokine storm, iNOS, TREM1, macrophage classical activation. In contrast to 2 and 6 hours (mainly characterized by activation of acute phase response and IL-17 signaling), transcriptional profiles of 24 hours showed significant inhibition of cell cycle regulation, purine and pyrimidine nucleotide biosynthesis, oxidative phosphorylation, as well as base excision repair pathway. Overall, these data seem to support a potential mechanism by which *Brucella* is able to replicate within macrophages and establish a chronic infection.

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C37 Neurophage: molecular engineering of phage nanoparticles for non-invasive neuronal photostimulation

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The M13 phage has emerged as a versatile nanocarrier with a wide range of innovative nanobiotechnology applications. Its distinctive filamentous shape, coupled with the arrangement of different coat proteins along its structure, provides an exceptional cargo capacity for genetically fused or chemically conjugated molecules. In addition, the intriguing and unexplored characteristic of M13 phage to cross the Blood-Brain Barrier (BBB), makes it a promising delivery agent for the treatment of different neurological diseases, overcoming challenges in the biomedical field.

The mechanisms that enable M13 to cross the BBB were investigated *in vitro* on 2D and 3D BBB models. Furthermore, the high cargo capability and ease of genetic handling were exploited to enhance its crossing ability by displaying BBB interacting peptides in fusion with the phage's major coat protein (pVIII).

Additionally, nanobodies were displayed on the phage's minor coat proteins (pIII), to enable the retargeting of the nanobiotechnological platform towards specific cell populations. As proof of concept, an anti-ALFA tag nanobody expressed in fusion with the pIII protein allowed the specific targeting of the phage to engineered neurons expressing a synthetic ALFA-transmembrane protein.

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After validation of the BBB crossing ability and targeting specificity, further modifications are currently being introduced to this phage vector platform to target various central nervous system receptors implicated in pathological pathways. These modifications will involve genetic manipulation of mice and chemical conjugation of photovoltaic materials to the phage, with the overarching goal to demonstrate the non-invasive photostimulation of denervated neurons by the Neurophage nanobot.

C38 Exopolysaccharides released by vaginal *Lactobacillus* strains: chemical characterization and modulation of biofilm formation

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Exopolysaccharides (EPS) are high molecular mass polymers produced by microbial cells and are involved in cell adhesion, auto-aggregation and prevention of pathogen growth. Little is known about EPS produced by vaginal lactobacilli, although anti-inflammatory and antitumoral effects have been proposed.

EPS released by vaginal *Lactobacillus* strains (*Lactobacillus crispatus* BC1, BC4 and BC5 and *Lactobacillus gasseri* BC9, BC12 and BC14) were isolated and chemically characterized by LC-UV and LC-MS to determine monosaccharide composition. The capability of EPS to modulate biofilm

formation was tested towards beneficial vaginal lactobacilli and pathogens commonly responsible for vaginal infections and assessed by MTT reduction assay and Cristal Violet biomass staining.

EPS turned out to be heteropolysaccharides, D-Mannose and D-Glucose are the most abundant monomers in *L. crispatus*- and *L. gasseri*-derived EPS, respectively. EPS promoted the formation of biofilms of beneficial *L. crispatus*, *L. gasseri* and *Limosilactobacillus vaginali*s strains, in a dose-dependent manner. Notably, EPS ¬¬mostly stimulated the biofilms of the same producer species rather than that of other species. Conversely, EPS significantly reduced biofilms of bacterial (*Escherichia coli*, *Staphylococcus* spp. *Enterococcus* spp., *Streptococcus agalactiae*) and fungal (*Candida* spp.) pathogens. *L. gasseri*-derived EPS showed the best anti-biofilm profile.

The present study provides insights into the functionality of EPS released by the most preponderant *Lactobacillus* species in the vaginal microbiota and supports their employment as a therapeutic/preventive strategy to counteract vaginal infections.

C39 Silencing of *speG*, encoding the spermidine acetyltransferase, contributes to succesfull *Shigella* infection of macrophages

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Shigella, the causative agent of bacillary dysentery, is a human pathogen evolved from its innocuous ancestor *Escherichia coli* through the acquisition of virulence determinants and the loss of genes which negatively interfere with bacterial fitness within the host. The so-called pathoadaptive mutations, that characterize the latter event, have also affected metabolism pathways of polyamines, small molecules found in all cells and involved in microbial pathogenesis. Indeed, *Shigella* has lost *speG*, a crucial gene which encodes spermidine acetyltransferase, an enzyme catalysing the conversion of spermidine into the physiologically inert acetylspermidine. What is the evolutionary advantage for *Shigella* in preventing the expression of a functional SpeG protein?

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In order to evaluate the extent of *speG* inactivation in *Shigella* and to understand the role of polyamines in the *Shigella*-host interaction, we evaluated the infectious process of a *Shigella speG*-complemented strain within macrophages. Data from THP-1 cell infection revealed that restoring acetylspermidine production is detrimental for both macrophages and *Shigella* indicating that inactivation of the *speG* gene is a key determinant in the pathogenicity strategy adopted by *Shigella*.

C40 The VHS phosphorylation is the key factor for the modulation of the vhs-activity during HSV-1 replication

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The model "host shut off" adopted by virion host shut off (VHS) protein of Herpes simplex type 1 (HSV-1) represents an immune evasion mechanism which affects the best-characterized component of the innate immunological response, protein kinase R (PKR). Previous our findings reported also viral tegument proteins U_S3 and U_L13 control the activation of PKR during HSV-1 infection with a mechanism which is probably related to the reciprocal regulation between viral

tegument proteins. Indeed, we found that VHS exerts its RNAse activity on Us3, unlikely to VHS mutant protein, and limits the accumulation of U_S3 protein. Besides, a shifted high molecular weight VHS band was detected in western blot analysis following U_S3 transfection [Pennisi R. et al.,2020]. Therefore, the aim of our study was to determine whether Us3 kinase activity was responsible for the VHS phosphorylation and individuate the phosphorylation site on VHS sequence. 293T cells were transfected with K220mutantUs3 plasmid in which the kinase activity is abrogated. The results reported that the mutation on kinase activity of U_S3 annuls the accumulation of VHS at high molecular weight demonstrating that the kinase activity of U_S3 is necessary to phosphorylate VHS. Besides, site directed mutagenesis on VHS indicated that U_S3 phosphorylates VHS on threonine/serine 141-142 residues. Collectively, our findings reveal a regulatory role between viral proteins which can help virus to prevail on the host immune response.

C41 Nasal microbiota, virus and air pollution interaction can explain differences of bronchiolitis outcome on host

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The role of pollutant exposure in worsening pathological outcomes is becoming ever more relevant. In the case of pediatric bronchiolitis, hospitalization peaks correlate with particulate matter (PM) pollutant exposure, but the players of this effect are still unknown. We hypothesize that exposure to PM can induce nasal microbiota alterations, which can determine host response diversities to infectious agents, leading to aggravated pathological states. To test our hypothesis we analyzed microbiota composition via 16S rRNA sequencing on nasal swabs from 103 bronchiolitis cases and 46 healthy controls (HC). Adjusting for both PM10 and PM2.5 exposure, Dolosigranulum genus abundance was decreased and Haemophilus increased in cases; furthermore a significant positive correlation was observed between Haemophilus and PM 10 exposure level at 3 weeks before sampling (p-value of interaction= 0.0489). Subsequent shotgun metagenomics analysis on 26 Respiratory Syncytial Virus (RSV) positive bronchiolitis and 19 HC, recapitulated 16S results and highlighted 25 functions, several related to *Haemophilus influenzae* (Hi) specie, present only in patients. A zebrafish model to study the immunomodulatory effects of short-term exposure to PM via Hi-derived extracellular vescicles (EVs) was set up, observing a strong pro-inflammatory potential in zebrafish embryos. Finally, preliminary *in vitro* data suggest that laryngeal epithelium cells pre-treatment with Hi EVs can advantage RSV infection. Altogether, these findings show that bacterial EVs may be involved in microbiota-host immune response interaction, modulating airways inflammation and promoting viral infections. This enforces the role of microbiota as key mediator between pollutant exposure and host health.

C42 Upper and lower respiratory tract microbial biomarkers associated with healthy pigs

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A decade of microbiome studies has linked respiratory disease in pigs, collectively named Porcine Respiratory Disease Complex (PRDC), to an alteration in the respiratory microbial community, especially of the upper respiratory tract. We aimed at identifying bacterial and fungal biomarkers in the upper and lower respiratory tract of pigs associated with the animal health status. During a PRDC outbreak in a conventional Danish pig farm, 35 pigs were sampled by nasal and tracheobronchial swabbing. Bacterial microbiota was analysed by 16S rRNA gene sequencing, and the fungal microbiota of nasal samples was additionally profiled using ITS rRNA gene sequencing. Sampling, extraction and run controls were also included. Data analysis was performed in R. Upon clinical examination, 25 animals presented clinical signs of PRDC (coughing), whereas 10 were healthy. Although no differences were observed in microbial diversity and richness between healthy and diseased pigs, differential abundance and linear discriminant analyses identified different taxa that were significantly associated with healthy individuals (p-value <0.05). One ASV assigned to *Pediococcus pentosaceus*, a member of lactic acid bacteria with known probiotic effects, was invariably correlated to the nose and trachea of healthy pigs. In the nose, three fungal species were associated with healthy animal, including the probiotic candidate *Wickerhamomyces anomalus* (previously *Pichia anomalus*). In the trachea, *Aerococcus viridans* and *Kurthia spp*. were correlated with healthy animals. We identified microbial taxa significantly associated with healthy respiratory tract, which can be further investigated as probiotic candidates for PRDC prevention.

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C43 Exploiting phages–bacteria co-evolution to overcome phage resistance and to ease the selection of new phage particles

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Antibiotic resistance is one of the major threats of modern medicine. The limited conventional treatment options against multi-drug resistant bacteria, as well as the paucity of new effective antibiotics, is leading to the need of developing novel strategies, such as the therapeutic use of bacteriophages. However, it is necessary to combine a variety of phages to avoid the therapeutic inefficacy due to the possible resistance emergence to single particles. Here, we describe a novel bacteriophage, named GP-7, which was isolated from hospital wastewaters collected in Tuscany following standard methods. GP-7 was obtained using the *K. pneumoniae* BO-FR-1 strain, a member of sequence type 258 resistant to the infection of the previously characterized phage phiBO1E. Host spectrum was determined by spot-test and efficiency of plating techniques. Physiological features, including stability to pH and temperature changes was assessed. The

kinetic of infection was defined by the one-step growth curve method. Phage genome was characterized by a next generation sequencing approach and bioinformatics analysis. GP-7 is myovirus with a strictly lytic cycle and exhibiting a narrow host spectrum restricted to its indicator strain and other *K. pneumoniae* phage-resistant mutants. The phage maintains its full infectivity between pH 4 and 11, and it is also stable after 1 h at 60° C. The infective cycle is characterized by a latency period of 25 min and a burst size of 45 particles. Results from this study could be of interest for the rationale design of phage cocktails to be used for therapeutic applications.

C44 *Neisseria meningitidis* HrpA interacts with dynein motor protein, affects internalization into the host cell and pyroptotic pathway

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Neisseria meningitidis (the meningococcus) is a Gram-negative bacterium transiently residing in the human nasopharynx of healthy subjects. In some of these individuals, it breaches the mucosal barrier, moreover, it can cross the blood-brain barrier causing meningitis. Among virulence factors, secreted proteins of two-partner secretion (TPS) systems could be targets for vaccine and/or treatment development. Using yeast two-hybrid system we found that HrpA (hemagglutinin/hemolysin-related proteins A), the secreted portion of the meningococcal TPS HrpA/HrpB, interacts with dynein light chain (DYNLT1), a motor protein moving along microtubules. To investigate the role of this interaction, we infected three cell lines with strains B1940 or mutated B1940Ω*hrpA*. Confocal microscopy showed only a few mutated bacteria internalized in neuronal cells compared to wild-type ones, moreover, these were distributed in the cell body and neurites while most of the mutated meningococci were located in the cell body. Western blot analysis revealed that pyroptotic pathway was activated following infection. This is a pro-inflammatory programmed cell death that relies on caspase-1 for the canonical route, caspase-11 for non-canonical route, both leading to cleavage of gasdermin-D (GASDMD) or on caspase-3 and gasdermin-E (GASDME). Active forms of caspase-1, caspase-11 and GASDME were detected only in infected cells and their expression was reduced when the infection was performed with B1940Ω*hrpA* bacteria; the active form of caspase-3 was overexpressed after infection while GSDMD showed no activation. In conclusion, our results showed that HrpA is important for the internalization and distribution of bacteria inside neuronal cells and that HrpA-DYNLT1 interaction affects pyroptotic pathway.

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C45 SARS-CoV2 Spike Protein Can Contribute To Extracellular Traps Production By Myeloid-Derived Suppressor Cells From SARS-CoV-2 Infected Patients

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Polymorphonuclear Myeloid-derived suppressor cells (PMN-MDSC) are increased during SARS-CoV2 infection, they are involved in the inhibition of SARS-CoV2 specific T-cell response and have been proposed as an early marker of COVID-19 fatal outcome. Neutrophil Extracellular traps (NET) are extracellular, web-like structures composed of cytosolic and granule proteins assembled on a scaffold of DNA. NET protect against infection, but they are also implicated in the pathology associated with a growing number of immune-mediated conditions.

In this work, we evaluated the role of PMN-MDSC from COVID-19 patients in the formation of extracellular traps (ET).

PMN-MDSC were purified from PBMC of SARS-CoV2 infected patients and stimulated with platelets-depleted and platelet-rich plasma (PL and PRP respectively) or with SARS-CoV2 Spike protein. ET formation was evaluated by confocal microscopy and quantified by pico488 fluorescence. PMN-MDSC-induced primary endothelial cell apoptosis was evaluated by flow cytometry.

PMN-MDSC from infected patients were able to extrude ET upon PRP and PLP stimulation. Differently, PRP from healthy donors was not able to induce ET. We also found that also other factors than platelets are involved in ET extrusion. Indeed, PMN-MDSC treatment with SARSCoV-2 Spike protein induced ET by PMN-MDSC, indicating that the virus itself could contribute to ET formation. We also found that PMN-MDSC induced endothelial cell apoptosis in an ET-independent manner.

We demonstrated that PMN-MDSC can extrude ET, and the spike protein may have a role in this. These data highlight a new role of PMN-MDSC that might participate in thrombotic events observed in severe COVID-19.

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C46 De novo design of antimicrobial and antibiofilm peptides starting from desert truffle *Tirmania pinoyi* peptides

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With the aim of discovering new routes in the research of antimicrobials, we focused on polypeptide-enriched extracts derived from edible desert truffle mushroom *Tirmania pinoyi*. The extracts showed an interesting activity with MIC=50 µg/mL against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 15442. Through mass spectrometry analysis (RP-HPLC/nESI-MS/MS) the following eight novel peptides FDVVPKTAANFRAL, AVTVGASTLADERA, FLVGGASLKPEF, VARIFAVFNDTF, HLVDEPQNLLK, LGEYGFQNALLR, FAVNGGCAKET, SREDLHPKL were detected. To characterize them online websites were used: IAMPpred, DPBAAS, Cell-PPD, ToxinPred, HemoPI, PeptideCutter and HLP. The analysis indicated that some peptides showed negative or neutral charge, hydrophobic ratio between 42% and 67%, Boman Index < 2.25 kCal/mol. According to the "APD3: Antimicrobial Peptide Calculator and Predictor" tool of the Antimicrobial Peptide Database (APD) similarities (around 30-40%) with known antimicrobial peptides (AMPs) identified in amphibians were also detected. In contrast, the predicted antimicrobial, antifungal and antibiofilm activity was not significant.

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In order to improve biological and physico-chemical properties, the sequences of natural peptides were modified using APD3, by replacing some hydrophilic and negative charged amino acids with hydrophobic and positive ones. The derivative sequences (GWDVVPKTWWKFRAL, KWTWGASTLAKKRA, FLRGGWSLKPKF, KWRIFWVFNKTF, HLVKRWQNLLK, KGKYRFWNALLR, FARWGGCAKRT, SRKWLHPWL) showed net positive charge between +2 and +4, hydrophobic ratio between 42% and 48%, Boman Index < 2.25 kCal/mol and high stability. Moreover, the predicted antimicrobial, antifungal and antibiofilm activity was high, without toxic or hemolytic effects.

In conclusion, bioinformatic analysis has demonstrated that novel peptides discovered in *T. pinoyi* may be considered new platforms for the design of novel antimicrobial and antibiofilm peptides to counteract multi-drug resistant pathogens.

C47 Linking the bacterial endophytic communities to the essential oil of the medicinal plant *Origanum vulgare* L.

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Medicinal and aromatic plants represent a natural source of bioactive molecules. In particular, antibacterial, antifungal and antiviral activities have been reported for the medicinal plant *Origanum vulgare* L. and its essential oils (EOs). Recent advances have been made regarding metabolite production by plant microsymbionts, showing that they may produce different types of bioactive molecules and/or modulate plant metabolite synthesis.

The aim of this work is to characterize the bacterial endophytic communities associated with *O. vulgare*, to select a collection of bacteria able to synthesize antimicrobial molecules and to understand if the aroma profile of the plant EO might be influenced by the presence of the endophytes and/or if some EO compounds might be synthesized by the endophytes themselves.

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To this purpose, endophytic bacterial communities were isolated from different *O. vulgare* anatomical parts and the EO was hydro-distilled from the same plant. The production of antibacterial secondary metabolites by endophytic bacteria was evaluated through antagonistic tests. Some of the isolates were able to inhibit the growth of multidrug-resistant bacteria, through the synthesis of diffusible, but also volatile organic compounds, which were characterized by SPME/GC-MS. Some of the identified compounds were also found in the EO chemical profile, suggesting the involvement of bacterial endophytes in the shaping of plant metabolome. The set up of an axenic *in vitro* model of *O. vulgare* might shed light on the close metabolic relationship between the plant and its phytobiome.

C48 Metabolic adaptation of *Mycobacterium tuberculosis* to iron starvation

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At the onset of infection, the host triggers a response called "nutritional immunity" to halt pathogen replication. The nutritional immunity response consists of altering the availability of metals (micronutrients) to either starve or intoxicate pathogens. To survive this condition and be able to persist in host tissues, successful pathogens have developed high-affinity metal-binding molecules and efficient efflux pumps. Despite its ubiquity and importance in host-pathogen interactions, the effect of changes in metal availability has been poorly characterised, in comparison with

macronutrients, such as carbon and nitrogen sources.

Here, we investigate the effects of Fe^{3+} starvation on the central carbon metabolism of *Mycobacterium tuberculosis* grown in the presence of glucose and glycerol carbon sources. Using carbon stable isotopes (^{13}C) coupled with mass spectrometry we found that Fe^{3+} starvation causes a remodelling of the central metabolism through three specific mechanisms: i) reduction of Krebs cycle activity; ii) activation of anaplerosis to produce malate; iii) increased secretion of malate and pyruvate to maintain the proton motive force.

An in-depth analysis of published and new (presented here) transcriptomic data suggests that growth-arrested iron-starved *M. tuberculosis* induces *de novo* biosynthesis of trehalose. Accumulation of trehalose has been observed in growth-arrested acidic pH-exposed *Mycobacterium smegmatis*, and it has been suggested that it can be used once the pH becomes neutral. We believe that a similar mechanism may occur in growth-arrested iron-starved *M. tuberculosis*.

C49 Antimicrobial activity of *Catha edulis* leaves extracts and its endophytes against soil bacteria

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Plants can control and appose stress on their bacterial phytopathogen in different ways. We investigate the antimicrobial potential of Khat leaves and its endophytes. Khat (*Catha edulis*) is a perennial shrub well known for its psychologic activity, causing a mildly stimulating euphoric, amphetaminic like effect. Khat leaves have been consumed for centuries by people living at the horn of Africa and the Middle East. In addition to these canonical uses, khat also played important roles in traditional folklore medicine, used to treat common illness such as stomachache diarrhea, and pneumonia. We extracted khat leaves, using either water, methanol, acetone, and hexane as solvents. The antimicrobial tests were performed using the Kirby-Bauer Disk Diffusion Susceptibility tested against *Escherichia coli* M4, *Bacillus thuringiensis*, S*taphylococcus aureus* and the phytopathogen *Acidovorax citrulli* M6, *Xanthomonas campestris pv. vesicatoria str. 85-10* and *Pseudomonas* syringae DC3000 bacterial strains. All solvents yielded extracts with antimicrobial properties, but methanol and acetone extracts displayed the strongest antibacterial effects. Heating methanolic extracts to 100°C did not abolish the antimicrobial activity. We isolate seven endophytes from the Khat and will present their antimicrobial activity against soil bacteria.

Concluding, khat leaves possess antimicrobial activity, that resist heating to 100°C. Antibacterial compounds, readily extracted into methanol. khat leaves contains endophytes that contribute to the antimicrobial activity of the plants.

Keywords

Khat, traditional medicine, antimicrobial compounds, leaves extraction, endophytes

C50 Immunomodulation of human macrophages by probiotic *Lactiplantibacillus plantarum* of plant origin: focus on their postbiotic potential

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As a probiotic, *Lactiplantibacillus plantarum* (Lp) interacts with the gut microbiota and host cells, thereby exerting a beneficial impact on host functions, mostly through immune-regulatory activities. In this study we assessed the immunomodulatory properties of Lp-derived postbiotics, both as molecules secreted into the culture medium (i.e., cell-free culture supernatants, CFSs), and as heat-inactivated cells on human macrophages.

Gene expression analysis and enzyme-linked immune assay revealed a strong basal induction of TNF-α gene transcription and secretion, particularly by CFSs from 10A, 11A and CB56 strains, with an attenuated transcriptional activation and secretion of both TNF-α and IL-8 in response to a pro-inflammatory stimulus. Furthermore, postbiotics from Lp 10A and UFG121 induced a high IL-10/IL-12 transcript ratio in the macrophages compared with untreated control, which mirrored the ratio of the corresponding secreted cytokines, supporting a potential anti-inflammatory profile for these strains. Compared to CFSs, heat-inactivated cells showed a higher anti-inflammatory effect. Overall, the investigated strains are promising probiotic candidates, whose postbiotics exhibit immunomodulatory properties that may be relevant for biomedical applications.

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C51 Granuloma Like Structure (GLS) model for evaluation of antitubercular drug efficacy

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Granuloma is a multicellular host-defense mechanism that forms in response to external pathogens. Its primary function is to contain the infectious agent by encapsulating it in a limited area, preventing its spread and enhancing the immune system's response. The granuloma is an organized three-dimensional structure of macrophages, epithelioid cells, and multinucleated giant cells, surrounded by a ring of lymphocytes. *Mycobacterium tuberculosis* (*Mtb*) inside the granulomas is exposed to a peculiar environment characterized by limited nutrients, oxygen, and restricted drug penetration. Consequently, sensitivity to drugs might be different from that shown

extracellularly or in a macrophage monolayer.
To mimic this microenvironment faced by *Mtb*, an *in vitro* model (Granuloma-like structure - GLS) was developed using human peripheral blood mononuclear cells (PBMCs). GLS represents a promising assay to study early events in host-pathogen interactions and to evaluate the drug efficacy in this infection stage.
We are optimizing and characterizing this system to understand its potential compared to the conventional macrophage monolayer assay for drug activity against *Mtb*. Additionally, we are currently developing an agent-based *in silico* model to simulate interactions between drugs, host cells, and *Mtb*. This model aims to provide complementary scenarios that have the potential to enhance the evaluation of drug activity.
The complexity of the interaction between *Mtb* and macrophages should indeed recapitulate the *in vivo* features, providing additional data on the activity of drug candidates and drug combinations to evaluate in clinical trials.

This work was supported by the Innovative Medicines Initiatives 2 Joint Undertaking (grant No 853989).

C52 From virulence factors to drug development: MbtI as effective *Mycobacterium tuberculosis* target

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Tuberculosis, the infectious disease caused by *M. tuberculosis* (*Mtb*), is still nowadays among the top ten causes of death worldwide, and the first caused by a microorganism. Therefore, the development of novel antitubercular drugs is an urgent need, due to the spread of multi-drug resistant and extensively drug resistant strains. One of the possible strategies to overcome this problem could be the development of novel drugs focusing on virulence factors instead of essential proteins.

Iron is an essential cofactor for many *Mtb* enzymes and it is necessary for the bacterial survival and infection establishment. *Mtb* chelates the iron from the host thanks to its peculiar siderophores known as mycobactins. MtbI is an enzyme involved in the first step of mycobactin biosynthesis, and it is proved to be an effective druggable target.

In our previous studies, we successfully expressed and purified MbtI in recombinant form, and an appropriate fluorometric enzymatic assay was set up. Subsequently, we identified different classes of MbtI inhibitors, among which the phenylfuran-carboxylate structure revealed to be very effective scaffold against this enzyme. However, the potent activity found against the enzymatic activity does not translate in a similar potency against mycobacterial growth.

In this work, we report the characterization of novel derivatives, with increased lipophilicity to facilitate the passage through the mycobacterial membrane to reach its target, showing improved antimycobacterial activity thus representing new leads for the development of effective anti-virulence compounds.

C53 Microbiome variations at the clams-sediment interface may explain changes in local productivity yields of the clam *Chamelea gallina* in the North Adriatic Sea

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Chamelea gallina is an ecologically and economically important marine species in the Northwestern Adriatic Sea, which is currently suffering from occasional, and still unexplained, widespread mortality events.

In order to provide some glimpses in this direction, this study explores the connections between microbiome variations at the clam-sediment interface and the nutritional status of clams collected at four Italian productive sites along the Emilia Romagna coast, with a different incidence of mortality events, higher in the Northern sites and lower in the Southern sites.

According to our findings, each productive site showed a peculiar microbiome arrangement at the clam-sediment interface, with features that clearly differentiate the Northern and Southern sites, with the latter also associated with a better nutritional status of the animal. Interestingly, the *C. gallina* digestive gland (DG) microbiome from Southern sites was enriched in some growth-promoting microbiome components, capable of supplying the host with essential nutrients and defensive molecules. Furthermore, in experiments conducted under controlled conditions in aquaria, we provided preliminary evidence on the prebiotic action of sediments from the Southern sites, allowing to boost the acquisition of the previously identified growth-promoting components of the digestive gland microbiome by clams from the Northern sites.

Taken together, our findings may help define innovative microbiome-based management strategies for the preservation of the productivity of *C. gallina* clams in the Adriatic Sea, due to the identification and maintenance of a probiotic niche at the animal-sediment interface.

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C54 Characterization of anti HSV-1 activity of pistachios extracts on permissive and nonpermissive cells to viral infection

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Pistachio nuts represent a rich source of bioactive compounds including tocopherols, phylloquinone, carotenoids, chlorophyll, and flavonoids, responsible for the anti-inflammatory, antimicrobial and antioxidant effects [Mandalari G. et al. 2022]. The aim of this study was to evaluate the antiviral effect of natural (NRRE) and roasted unsalted (RURE) pistachio kernels, obtained by two different extraction methods with or without n-hexane, on permissive and nonpermissive cells to HSV-1 infection. Thus, epithelial VERO cells and human monocytic cells THP-1 were employed to verify the antiviral activity of both extracts. Prior to monitor the effect of NRRE and RURE on

viral replication, dose and time-dependent cell viability assay was performed. Thus, non-toxic concentrations of NRRE and RURE extracts were used on cell lines to evaluate the viral DNA accumulation and the viral plaques formation. The results report that both extracts exhibited a significant inhibitory activity on VERO infected cells at 0.6 mg/mL and in particular, the mixture extracted with n-hexane determined a significant reduction of plaque numbers. Besides, we found that NRRE and RURE n-hexane interfered with the production of viral genome compared with the untreated HSV-1. Similarly, we measured the intracellular virus production in THP-1 cells pre-treated with NRRE and RURE n-hexane and infected with HSV-1 by plaque assay and showed a strong reduction at both concentrations which matches with the viral DNA reduction following treatment. These findings demonstrate the anti-herpetic properties of pistachio extracts. Further studies are required to investigate the mechanisms involved.

C55 Exploiting dispirotripiperazines as a novel strategy to fight *Pseudomonas aeruginosa* infections in cystic fibrosis

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Pseudomonas aeruginosa chronic pulmonary infections still represent a major cause of death for patients with cystic fibrosis (CF). Indeed, novel effective antimicrobial treatments are lacking, and even the recent introduction of the CFTR modulator therapies appears to have a negligible impact on the clearance of airway infections. In this context, preventing host-pathogen interactions is a promising strategy to block bacterial lung colonization, also decreasing the need for prolonged antibiotic courses. PDSTP is a non-toxic dispirotripiperazine-based compound, able to impair the adsorption of many viruses to the mammalian cell surface by interacting with heparan sulphate glycosaminoglycans. Since *P. aeruginosa* can recognize the same surface receptors, the anti-adhesion ability of this compound was demonstrated *in vitro* using different human lung epithelial cell lines. Additionally, PDSTP impaired *P. aeruginosa* biofilm formation *in vitro*, as shown by confocal microscopy, and in *ex vivo* pig lung biofilm inhibition assay. By means of synergy and time-kill assays the compound also exhibited a significant ability to improve the efficacy of clinically relevant antibiotics against *P. aeruginosa* CF isolates. Finally, the synergistic activity of PDSTP was validated *in vivo* using a *Galleria mellonella* infection model. Altogether, these data establish PDSTP as a promising drug candidate for the management of *P. aeruginosa* infections in CF, capable of either interfering with the initial bacterial colonization of the lung or enhancing the efficacy of current antibiotic therapies.

This work was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project PE00000007, INF-ACT).

C56	**Diffusible Signal Factors (DSFs) repress Shigella virulence by binding to and hindering the action of its master regulator VirF**

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Shigella spp. is a gram-negative bacterium that causes bacterial dysentery and shigellosis. The VirF protein, encoded by the virF gene and classified as an AraC-like transcription factor (TF), is a key regulator of the virulence phenotype. VirF activates downstream genetic determinants of virulence by inducing *virB* transcription. Like other members of the AraC-XylS family of proteins, VirF has a "jelly-roll" module, a hydrophobic pocket that accommodates intestinal molecules such as saturated and unsaturated fatty acids, as well as members of diffusible signaling factors (DSFs), a rare class of fatty acids able to work as autoinducers. Our research has demonstrated that two representative DSF molecules, namely DSF and BDSF, directly interact with the jelly-roll module of the VirF protein, thereby causing inhibition of transcriptional promoter activity. Furthermore, DFS / BDSF-mediated repression is sufficient to significantly hinder *Shigella* invasion into host cells and their subsequent proliferation. These results suggest that the DSF molecules tested exert a significant antivirulence effect on *Shigella* and, overall, our study provides valuable insights into the structure and function of the VirF regulator and its interaction with inhibitory ligands. This knowledge opens up new possibilities for designing or selecting molecules that can serve as antivirulence drugs in the treatment of shigellosis.

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C57	**Development of immuno-conjugates magnetic graphene beads for specific extraction of potential SARS–CoV-2 biomarkers from enriched Extracellular Vesicles**

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Increasing studies demonstrated that liquid biopsy is a minimally invasive technology for the detection of molecular biomarkers in blood and other body fluids. Among these, the exosomes are important components of the liquid biopsy and can serve as carrier of proteins and nucleic acids (Cordaro A. et al., 2020). The aim of this study was to develop a vitro exosomes-enrichment system for the molecular characterization of the circulating exosomes upon SARS–CoV-2-binding on cellular surface. The SARS–CoV-2 binding-infection was reproduced in vitro by producing SARS-CoV-2 S-pseudotyped particles (SARS-Spp) expressing the Spike glycoprotein. SARS-Spp was produced on HEK-293t cells by simultaneous transfection with lentiviral expression and packaging plasmids, luciferase reporter and Spike -encoding plasmids (Millet J.K.et al., 2016). Quantification of the infectivity of SARS-Spp was performed by a luciferase activity assay on VERO cells. A549-ACE cells were transduced with SARS-Spp, after 48h the released-exosomes were purified by ultracentrifugation approach and characterized by western blot analysis of main exosomes target proteins. Next, magnetic beads were functionalized at 4°C with CD9 antibody, commonly used as marker for exosomes and incubated with SARS-Spp-transduced A549-ACE-derived exosomes. Preliminary data validated the success of conjugation protocol. Immuno-

conjugates magnetic graphene beads can represent a useful tool to search for new disease markers in body fluids by non-invasive method.

C58 Structure and regulation of the *hemO* gene cluster for heme uptake in *Acinetobacter baumannii*

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The scarcity of available iron in human fluids encountered by pathogens during infection is part of a non-specific host defense mechanism known as nutritional immunity. Most iron in the human body is stored in the heme-prosthetic group, primarily in hemoglobin. *Acinetobacter baumannii* possesses two heme acquisition systems, namely HemT and HemO. The *hemO* cluster is composed of two operons and two monocistronic genes. One operon consists of two genes encoding an extracytoplasmic function (ECF) sigma factor and an anti-sigma factor, while the second one is composed of four genes coding for the hemophilin secretion modulator (*hsmA*), a TonB-related protein, a heme oxygenase, and a hypothetical protein. The monocistronic genes encode the TonB-dependent receptor, *hphR*, and the hemophilin, *hphA*.

Gene expression analyses were carried out to characterize the regulation of the *hemO* gene cluster. The gene expression analysis demonstrated that the ECF sigma factor, *hphR*, and *hphA* genes are regulated by iron, and they are expressed when *A. baumannii* is cultured in biological fluids like human serum, saliva, and urine. By expressing *in trans* the ECF sigma factor in *A. baumannii* ATCC 19606^T strains carrying the transcriptional fusions between the promoters of *hemO* cluster genes and *luxCDABE* operon, we also demonstrated that ECF sigma factor controls the expression of *hphR*, *hphA*, and *hsmA*. Finally, by using a *Galleria mellonella* larvae model of infection, we demonstrated that iron scarcity represents a cue that triggers the expression *hemO* cluster and enhances *A. baumannii* virulence.

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C59 The ESX-2 secretion system is involved in Mycobacterium tuberculosis resistance to host-related stress conditions

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Mycobacterium tuberculosis (*Mtb*), the causative agent of human tuberculosis, expresses five type VII secretion systems (ESX secretion systems, ESX-1 — ESX-5). While ESX-1, ESX-3, and ESX-5 have been shown to play crucial functions in mycobacterial physiology and virulence, ESX-2 remains poorly characterized, and its biological role is still unclear. Microarray data suggest that the regulation of the ESX-2 encoding *locus (esx-2)* might involve WhiB5, a mycobacterial-specific transcriptional factor induced under selected stress conditions (nutrient starvation) and required for *Mtb* reactivation in a murine model of chronic tuberculosis. In this study, the analysis of *esx-2* gene expression profiles showed an increased expression of: i) *esxC* and *esxD* (encoding for

predicted ESX-2 substrates) following incubation of *Mtb* in nutrient depleted media (phosphate buffer); ii) *espG*$_2$, *mycP*$_2$ (encoding for potential ESX-2 modulators), *esxC*, *and esxD* in *Mtb* cultures reactivated after hypoxia-induced *in vitro* dormancy. Consistently, a panel of *Mtb* ESX-2 mutant strains (*MtbΔESX2*, deleted for the *esx-2 locus*; *MtbeccC*$_2$*KO*, inactivated for the EccC$_2$ ATP-ase encoding gene; *MtbΔesxC*) showed reduced survival after incubation in phosphate buffer, and a delayed growth recovery following reactivation from dormant cultures, as compared to the wild-type strain. The corresponding complemented strains displayed survival ability and growth recovery comparable to the wild-type. These *in vitro* findings provide the first evidence of the potential role of ESX-2 in promoting *Mtb* viability under selected stress conditions. Further characterization of ESX-2 mutants in cellular and *in vivo* models will contribute to clarify the function of ESX-2 and its impact in host-pathogen interactions.

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