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P01

Heterogeneous Expression of Oxidative Stress-Associated Genes in Populations Recovering from β-lactam Exposure

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Bacteria, persistently subjected to changing environments, have evolved 'memory' mechanisms that induce distinct transcriptional profiles that can persist across generations [1,2]. Our study explores these mechanisms within *Escherichia coli* MG1655, subjected to intermittent exposure to a β-lactam antibiotic. Using fluorescent transcriptional reporters [3], we evaluated the gene regulatory dynamics that emerge in response to an anticipatory stimulus to identify genes with hysteretic behavior, many of which were linked to oxidative stress. We also employed time-resolved flow cytometry to analyze *oxyR*, a key oxidative stress regulator, finding a bimodal distribution in its expression after a stress-free recovery period. Notably, the subpopulation that maintained high *oxyR* expression levels displayed increased tolerance to multiple classes of antibiotics upon reintroduction of the antibiotic. This result suggests that bacterial populations could be implementing a bet-hedging strategy, with most of the population rapidly recovering from stress while a distinct subpopulation maintains a stress-adapted transcriptional profile that enhances their tolerance to future stressful encounters. Finally, we used a mathematical model and computer simulations to examine the impact of different stress inheritance mechanisms, providing insights into bacterial survival strategies in fluctuating environments.

[1] Bheda, P. Metabolic transcriptional memory. Molecular Metabolism 38, 100955 (2020).
[2] Govers, S. K., Mortier, J., Adam, A. & Aertsen, A. Protein aggregates encode epigenetic memory of stressful encounters in individual escherichia coli cells. PLoS biology 16, e2003853 (2018).
[3] Zaslaver, A. et al. A comprehensive library of fluorescent transcriptional reporters for escherichia coli. Nature methods 3, 623–628 (2006).

P02

From Arduous Stress to Adaptive Success: A systematic investigation of physiological adaptation of *Escherichia coli* facing harsh environmental changes (Poster)

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Bacteria continually experience heterogeneous and rapidly changing environments. To survive and thrive, bacteria must quickly sense and respond to environmental shifts [1, 2]. This requirement is exemplified in pathogens during host colonisation [3, 4]. To successfully transition from the intestine to the urinary track, uropathogenic *Escherichia coli* must rapidly switch from an energy-rich to an energy-poor nutritional environment with less sugars, metals and amino acids [5]. Similarly, *Mycobacterium tuberculosis* must adapt in more acidic and oxidative conditions when infecting alveolar macrophages [6, 7].

The adaptive response to such harsh environments is mediated by a multi-layered regulatory network [8–10]. Its underlying mechanisms range from long-term and slow changes in gene expression to rapid and transient post-translational modifications [11]. Physiological adaption often arises from the interplay of mechanisms at different regulatory layers [11, 12]. High-throughput technologies were integral to systematically unravel the regulatory connections in this intricate network, including transcription factor-target gene and protein-metabolite interactions [13, 14]. The mechanisms by which environmental changes propagate through the network and how adaption is orchestrated to ensure homeostasis remain however elusive.

In this poster, I will present our approach to systematically unravel and model the interplay between transcriptional regulation and metabolism by combining high-throughput metabolomics with genetic interference of transcription factor expression. Our strategy involves integrating metabolic profiles with additional phenotypic data (e.g. growth data) and existing knowledge of the regulatory network [13]. Through this integration, we aim to develop mathematical models that shed mechanistic insights into bacterial adaptation to harsh environments and that are able to predict new ways to interfere with essential pathogenic regulation during host infection.

[1] H. Link *et al.*, Nat. Methods **12**, 1091 (2015).
[2] O. Kotte, J. B. Zaugg, M. Heinemann, Mol. Syst. Biol. **6**, 355 (2010).
[3] T. M. Fuchs *et al.*, FEMS Microbiol. Rev. **36**, 435 (2012).
[4] A. A. Crofts *et al.*, Proc. Natl. Acad. Sci. U. S. A. **115**, E8968 (2018).
[5] C. C. Y. Chan, I. A. Lewis, Trends Microbiol. **30**, 1174 (2022).
[6] J. J. Baker, S. J. Dechow, and R. B. Abramovitch, Trends Microbiol. **27**, 942 (2019).
[7] P. Chandra, S. J. Grigsby, and J. A. Philips, Nat. Rev. Microbiol. **20**, 750 (2022).
[8] D. W. Erickson *et al.*, Nature **551**, 119 (2017).
[9] R. Tollerson, M. Ibba, J. Biol. Chem. **295**, 10434 (2020).
[10] M. Zampieri *et al.*, Nat. Commun. **10**, 3354 (2019).
[11] D. Ledezma-Tejeida, E. Schastnaya, U. Sauer, Curr. Opin. Syst. Biol. **28**, 100404 (2021).
[12] H. Link, K. Kochanowski, U. Sauer, Nat. Biotechnol. **31**, 357 (2013).
[13] V. H. Tierrafría *et al.*, Microb. Genomics **8**, (2022).
[14] K. G. Hicks *et al.*, Science **379**, 996 (2023).

P03

Thermodynamic study of the self-organization structures of *Bacillus subtilis* colonies

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During colony growth, bacteria commonly generate self-organization structures that resemble those observed in chemical systems. Such patterns stem from the non-linear interactions between microorganisms and the environment. Because bacterial communities can be understood as open chemical systems out of equilibrium, they can be studied according to the foundations of non-equilibrium thermodynamics and linear stability analysis [1]. Thus, this work implemented such an approach to investigate the bacteria's self-organized structures formed, motivated by observed patterns in *Bacillus subtilis*. To this end, a mathematical model based on a system of reaction-diffusion equations [2] was used to evaluate whether the nutrient concentration and viscosity of the semi-solid medium affect the formation of self-organization structures. The linear stability analysis and numerical simulations results showed that disk-like and rough round patterns emerge in nutrient-rich semi-solid media with high bacteria motility, where the colony growth occurs as a traveling wave whose shape and propagation velocity are constants. In turn, in semi-solid media with low bacterium motility, diffusion-limited aggregation, and dense-branching patterns emerge, which indicates that the active bacteria sub-population propagates like a pulse. Although this work focuses on the self-organization structures observed during the growth of *B. subtilis* colonies, the approach proposed here can also be applied in the study of dissipative structures found in colonies of other microorganisms, as well as in biological systems with different levels of organization. Moreover, the results of this study will be used as a starting point for discussing whether the analysis of living systems through non-equilibrium thermodynamics and the principle of maximum entropy production can provide insights for understanding biological phenomena.

[1] I. Prigogine, G. Nicolis, “Self-Organisation in Nonequilibrium Systems: Towards A Dynamics of Complexity”.In: Bifurcation Analysis, Springer, Dordrecht. 3 (1985).
[2] M. Mimura, H. Sakaguchi, M. Matsushita, Physica A. **282**, 283 (2000).

P04

Patterns and dynamics generated across spatial scales for the assembly of a synthetic microbial community

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Within natural environments, we rarely find bacteria living in isolation, but in association with other microorganisms. Despite all that is known about bacteria, the study of the assembly and consolidation of bacterial communities is still an open field. With this project, we will explore how the different initial conditions in which a community develops affect different spatial scales, generating patterns that may affect the final result. We will closely monitor a Bacillota community that was first isolated from a Basin in Cuatro Ciénegas, Coahuila, Mexico, in which each of its members has a different ecological role, and the stabilization and establishment of the community depends on inhibition interactions. In this work, we aim to understand the effect of densities and space in the metabolic interaction of this system, and we try to understand better the mechanisms that establish this particular community while disentangling how variable environments may affect their assembly, assessing their effects from the single cell level to whole populations.

P05

Abstract for poster presentation for 'Advanced School on Quantitative Principles in Microbial Physiology (smr3879)'. Title: Effect of Long-term frequent anoxic-aerobic cycling on a denitrifying community and its N_2O emission

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Nitrous oxide (N_2O) is a 300x stronger greenhouse gas than carbon dioxide (CO_2) and currently the main ozone-depleting compound [1]. N_2O can be produced as by-product during biological nitrogen removal (BNR) by microorganisms in wastewater treatment plants (WWTPs). During BNR, activated sludge is exposed to frequent cycling of anoxic-aerobic conditions to perform denitrification and nitrification. Denitrification normally only occurs under anoxic conditions, being both a source and a sink of N_2O [2,3]. Short-term effects of oxygen on denitrification have been previously studied, such as the inhibition of the N_2O reduction step [4]. Long-term adaptation mechanisms of denitrifying communities to anoxic-aerobic cycling are, however, still to be determined. Therefore, in this study, the effect of frequent long-term anoxic-aerobic cycling on two denitrification communities and their N_2O emission was investigated. Denitrifying organisms were enriched from activated sludge in two identical lab-scale reactors, continuously fed with nitrate (NO_3^-) as electron acceptor and a mixture of volatile fatty acids (VFAs) as electron donor and carbon source. Reactors were operated for 52 days under a low and high frequency anoxic-aerobic cycling, exposed to 4 and 32 cycles per day, respectively. Metagenomic data revealed that the dynamic conditions led to a complex denitrification community of which a large part contained the full set of denitrification genes. Surprisingly, aerobic denitrification was observed in both reactors throughout the entire operation, while dissolved oxygen concentrations were above 6 mg/L during aerobic conditions. This was likely an adaptation mechanism of the denitrifying community to the dynamic oxygen environment. As seen by others, the recovery of N_2O reduction activity by the community after oxygen exposure seemed to be slower than other denitrification steps, resulting in transient N_2O accumulation at the start of anoxia. Curiously, the N_2O emission rate in the high frequency reactor was similar between both phases and in the low frequency reactor this was even higher for the aerobic phase. A combination of multiple scenarios, such as an imbalance in denitrification due to differences in oxygen sensitivity of the enzymes, more electron competition or less abundance of potential N_2O reducers in the low frequency system, were possible explanations. Although from this study it was not possible to conclude what anoxic-aerobic configuration is more preferable for N_2O mitigation strategies in WWTPs, the work contributed to a better understanding of the impact of dynamic oxygen environments on a denitrification community.

[1] IPCC. Climate change 2014 synthesis report. *IPCC* (2014).
[2] M. M. Kuypers, H. K. Marchant, B. Kartal. Nat. Rev. Microbiol. **16**, 263-276 (2018).
[3] M. C. M. van Loosdrecht, P. H. Nielsen, C. M. Lopez-Vazquez, & D. Brdjanovic, Experimental Methods in Wastewater Treatment. vol. 15, pp. 9 781 780 404 752–9 781 780 404 752 (2016).
[4] T. Suenaga, S. Riya, M. Hosomi & A. Terada. Front. Microbiol. 9 (APR), 1-10 (2018).

P06

A dynamic genetic switch to induce phenotypic state transitions in *Escherichia coli*

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Integrating mechanistic mathematical models with controlled experiments has become increasingly relevant for gaining a deeper understanding of the complexity of cells and the organization of microbial communities [1]. Genetic engineering plays a central role in developing experimental microcosms that enable quantitative and mechanistic investigations into the impact of physiological and biological processes on microbial community dynamics [2,3]. This is typically achieved through DNA editing techniques, which allow the engineering of strains with prescribed interactions, such as cooperative or antagonistic behaviors. In addition to constitutive engineering of desired phenotypes through genome editing, *in vivo* DNA recombination allows researchers to dynamically transition cells between different phenotypic or physiological states. This capability enables researchers to investigate the impact of these transitions on the ecological and evolutionary dynamics of microbial communities.
For instance, in [4], this methodology was used to study somatic differentiation in an experimental model of multicellularity by inducing recombination of *lox* sites at low rates using the *Cre* recombinase in the eukaryote *Saccharomyces cerevisiae*. At present, however, generating such *in vivo* recombination events in prokaryotes is challenging due to the lack of a chemically inducible system that allows tuning the rate of transition. To address this, we developed and characterized a *Cre/lox* system that enables tunable, low-rate *in vivo* recombination in *Escherichia coli*. This system utilizes a repressible promoter with low leakage, incorporates a degradation tag on the *Cre* recombinase gene to reduce its activity, and can be introduced on either plasmids or the *E. coli* chromosome. Future applications include controlled and reproducible cells conversion in evolutionary and population dynamics experiments and the development of recombinase-based biosensors.

[1] S. Widder, R. Allen, T. Pfeiffer *et al*., ISME J **10**, 2557–2568 (2016).
[2] W. Kong, D.R. Meldgin, J.J. Collins *et al.*, Nat Chem Biol **14**, 821–829 (2018).
[3] B. Li, Y. Qiu, Y. Song, H. Lin, H. Yin, Environ Int. **131**, 105007 (2019).
[4] M.E. Wahl, A.W. Murray, Proc Natl Acad Sci USA **113**(30), 8362-7 (2016).

P07

Response of Polymicrobial Communities to Ribosome Binding Antibiotics

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Antibiotic tolerance mechanisms in microbial strains are usually investigated in clonal populations. However, many microbial infections emerge from body compartments that are colonized by several interacting strains. Such polymicrobial communities may respond differently to antibiotics [1]. The long-standing consensus that growth rate is positively correlated with susceptibility to antibiotics has been challenged by a recent study that showed that under ribosome-targeting antibiotic agents, the susceptibility is determined by the reversibility parameter of the antibiotics [2]. Namely, upon exposure to irreversibly binding ribosome-targeting antibiotics, it is the fast growing cells that die first (are more susceptible), however, for the irreversibly binding antibiotics - it is the slow growing cells that are more susceptible. The community-level response of polymicrobial communities to ribosome-binding antibiotics remains largely understudied. Here, we are presenting our work towards a unifying framework that shows quantitatively how the physiology of individual cells in polymicrobial communities determines the response to ribosome-targeting antibiotics. To achieve this goal, we use a synthetic microbial consortium composed of amino acid cross-feeding *Escherichia coli* auxotrophs [3]. In this community, we are using live-cell microscopy to quantify antibiotic susceptibility of each single cell in the community as a function of its growth rate, its position relative to other cells, and the concentration of different reversibly and irreversibly binding ribosome-targeting antibiotics. We also perform minimal model numerical simulations that model reversible dynamics of antibiotic binding to ribosomes and neighborhood-dependent growth rates of individual cells. The combination of computational and experimental techniques allows us to provide quantitative single-cell level insights into the relationship between the cross-feeding interactions, cell physiology, and susceptibility to ribosome-binding antibiotics in polymicrobial communities. Understanding these relationships holds potential for providing innovative clinical applications for achieving better control of polymicrobial infections.

[1] Tuomanen, Elaine, et al. "The rate of killing of Escherichia coli by β-lactam antibiotics is strictly proportional to the rate of bacterial growth." *Microbiology* 132.5 (1986): 1297-1304.
[2] Greulich, Philip, et al. "Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics." *Molecular systems biology* 11.3 (2015): 796.
[3] Dal Co, Alma, et al. "Short-range interactions govern the dynamics and functions of microbial communities." *Nature ecology & evolution* 4.3 (2020): 366-375.

P08

Molecular characterization and biological control potential of endophytic bacteria isolated from seeds of native tomato, paprika, and zucchini cultivars

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As a consequence of the constant population growth on planet Earth, there is an increasing need for food. According to some estimates, food production will have to double by 2050 to meet the needs of the world's population (1). To protect plants and increase their yields, chemical pesticides and fertilizers have been used for decades, causing damage to human health and the environment. The best way to overcome these obstacles is seen in the use of beneficial microorganisms, such as bacteria for the purpose of biopesticides, biofertilizers and bioremediators of polluted soil (2). The aim of this research was to isolate and molecularly identify endophytic communities of bacteria from the seeds of various Serbian indigenous varieties of tomatoes, paprika and zucchini. By cultivating bacteria, we have isolated 121 isolates. The largest number of isolates belonged to species of the genus *Bacillus*. After a series of different tests aimed at assessing the PGP characteristics and production of exoenzymes, the 44 best isolates were selected. Based on all the results, we have selected 9 isolates with the best shown properties. From tomato 2 isolates (*Bacillus zhangzhouensis* (GZ10) and *Paenibacillus taichungensis* (GZ24), from paprika 4 isolates (*Bacillus subtilis* (PPC8), *Paenibacillus polymyxa* (PPB4), *Bacillus pumilus/safensis/zhangzhouensis* (PPB13), *Bacillus halotolerans* (PPB30)) and from zucchini 3 isolates (*Bacillus zhangzhouensis* (TVF18 i TVN17) and *Bacillus subtilis* (TVN23)). Isolate GZ24 has stood out with the production of siderophores, while isolates TVN17, TVN23, PPC8, PPB4, PPB13 and PPB30 have showed a strong growth inhibition towards pathogenic fungi in dual culture tests.

Keywords: beneficial bacteria, endophytes, biological control, plant growth promotion

[1] Sessitsch, Angela, et al. "The contribution of plant microbiota to economy growth." *Microbial biotechnology* 11.5 (2018): 801.

[2] Kumar, Suresh, and Archana Singh. "Biopesticides: present status and the future prospects." *J Fertil Pestic* 6.2 (2015): 100-129.

P09

In silico evolution of bacteria metabolic networks shows the routes leading to metabolic innovations

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Genome-scale metabolic models (GEMs) are a powerful tool to understand and predict the metabolic status of bacterial species in different environmental conditions [1]. GEMs simulated using constraint-based models such as Flux Balance Analysis (FBA) [2] or Dynamic Flux Balance Analysis (dFBA) [3] make testable predictions and are used to answer different metabolic engineering, bioprocessing or ecological questions. Recently, GEMs have been used to model the evolution of metabolic networks [4]. Here, we ask the question: **how do complex metabolic innovations arise in bacteria (within and between species)?** We address this question using a constraint-based framework combining FBA/dFBA and the adaptive dynamics theory [5] to simulate the evolution of bacteria metabolic networks in an eco-evolutionary framework. We start with a GEM in a defined environment, called the resident. At each evolutionary time step, we create a mutant by adding or deleting one or several reaction(s) to the resident GEM corresponding to one or several mutation(s) chosen from a matrix containing all the known metabolic reactions in prokaryotes or a subset of this matrix, in line with [6,7]. Then, we perform FBA or dFBA on both the mutant and the resident, assuming that the environmental conditions are identical. The strain (resident or mutant) with the highest growth rate wins the competition and becomes the new resident, or, in a dFBA scenario, co-existence can happen. We then proceed to the next evolutionary time-step. By doing this evolutionary cycle iteratively, we can study the evolution of metabolic networks until an evolutionary equilibrium is eventually reached, accounting for ecological feedbacks. Taking the **cost of reactions**, **number of mutations (addition/deletion)**, **horizontal gene transfer** (many reactions added) into account, we show the different possible routes leading to metabolic innovations in bacteria under different environmental and genomic conditions.

[1] Terzer, M., Maynard, N.D., Covert, M.W., and Stelling, J. Wiley Interdisciplinary Reviews: Systems Biology and Medicine 1, 285-297 (2009)
[2] Orth, J.D., Thiele, I., and Palsson, B.Ø. Nature biotechnology 28, 245-248 (2010a)
[3] Mahadevan, R., Edwards, J.S., and Doyle, F.J. Biophysical journal 83, 1331-1340 (2002)
[4] Takano, S., Vila, J. C., Miyazaki, R., Sanchez, A., & Bajic, D. bioRxiv: 2023-04 (2023)
[5] Geritz, S.H., Kisdi, E., Meszena, G., and Metz, J. Evolutionary Ecology 12, 35-57 (1998)
[6] Zomorrodi, A.R., Islam, M.M., and Maranas, C.D. ACS synthetic biology 3, 247-257 (2014)
[7] Szappanos, B., Fritzemeier, J., Csörgő, B., Lázár, V., Lu, X., Fekete, G., Bálint, B., Herczeg, R., Nagy, I., Notebaart, R.A., Lercher, M.J., Pál, C., and Papp, B. Nature communications, 7(1), 11607 (2016)

P10

The role of randomness in bacterial stress response

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There is an immense effort to achieve a quantitative understanding of the cell's extremely complex network of biochemical interactions. While the ability to model the exact network is still out of reach, under certain conditions we can apply simplifying assumptions on its structure to achieve a computationally feasible system. It has been shown, that under a strong perturbation of antibiotics, E. Coli bacterium are driven into a "disrupted" state in which the recovery statistics of the colony are spread over many time scales[1]. This property gives the population a chance to "persist" an antibiotic treatment simply by the fact that a small number of the cells will arrest their growth for a very long time and thus evade the effects of the antibiotic.

These observations suggest that under specific conditions the cell dynamics can be governed by noise. On that account, we present modelling techniques that provide a deeper understanding of the underlying mechanism that drives the cells into the disrupted state. Our approach involves modelling the metabolic and regulatory network of the cell as a pseudo-random network on which quantitative conclusions can be drawn. The model shows a phase transition that exhibits physical properties which are consistent with experimental observations on the biological system.

Additionally, we analyse data of single cell RNA sequencing [2] performed on E. Coli bacteria under stress. Analysis of gene-gene correlations shows that the data exhibits structural features reminiscent of random data. We compare these results to both analytical and numerical calculations performed on models of gene-regulatory networks and their randomized counterparts. This analysis gives us a quantitative perspective on the role of randomness in the disrupted state of E. Coli and therefore contributes to a deeper understanding of the cell's ability to respond to an acute stress.

[1] Kaplan, Y. et al. Observation of universal ageing dynamics in antibiotic persistence. *Nature*, **600**, 290–294 (2021)

[2] McNulty, R. S. et al. Probe-based bacterial single-cell RNA sequencing predicts toxin regulation. *Nature Microbiology* (2023).

P11

How definitions of cellular volume impact the growth laws in models of cellular physiology

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The investigation of bacterial growth laws or the dependence of mass fractions of various cellular components on the cellular growth rate in different environments has been a subject of intense study in the recent past. However, what has not received sufficient attention is the relationship between cell volume and the molecular populations that make up the cell (the populations of both macromolecules and metabolites), and how this relationship affects the growth laws. We investigate this with existing coarse-grained mechanistic models of bacterial cells in a framework [1] in which the cell volume V is treated as a linear function of its internal molecular populations X_i: $V = \sum_i v_i X_i$, where the v_i are constants. The growth rate and molecular mass fractions are determined endogenously in this framework by applying an optimization principle (e.g., maximization of growth rate), or by positing an internal mechanistic regulatory mechanism (e.g., regulation of ribosomal fraction by the alarmone ppGpp). We find that the growth rate as well as the internal molecular mass fractions depend on the particular choice of the linear function V (i.e., on the coefficients v_i). Some choices reproduce the bacterial growth laws and others do not. In particular, a choice of the volume function for which the cell mass density is a constant (independent of the medium) does not naturally reproduce the growth laws. This suggests that the form of the volume function tightly constrains bacterial physiology and that experiments determining the coefficients v_i could be quite important.

[1] P.P. Pandey, H. Singh, S. Jain, Phys. Rev. E. **101**, 062406 (2020).

P12

On the physiology of Vibrio natriegens and the possible costs of growing “too fast"

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Vibrio natriegens is a marine bacterium with one of the fastest known growth rates: in optimal conditions its population can double in as little as 10 minutes. However, even though it is able to grow so fast, *V. natriegens* is not a dominant species in the marine environments where it was isolated from. To see whether this may arise from possible tradeoffs between fast growth with other physiological characteristics, we characterized various aspects of *V. natriegens*’ growth physiology, including yield, death, and lag times when switching between nutrients. We compared these results with those of *Vibrio splendidus sp. 1A01*, a closely related *Vibrio* species which grows 25% more slowly than *V. natriegens* even at its optimal growth temperature, which is 10°C lower than that of *V. natriegens*. No apparent physiological trade-off for *V. natriegens* could be identified, as *V. natriegens* performed at least as well as *V. 1A01* in all aspects examined. One ability *V. natriegens* lacks compared to *V. 1A01* is the degradation of chitin, a polysaccharide of N-acetylglucosamine (GlcNAc) that is common in the marine environment, even though *V. natriegens* grows better than *V. 1A01* on the monomer GlcNAc itself. Interestingly, When *V. natriegens* is introduced in a *V. 1A01* chitin culture, it strongly inhibits the growth of the whole population, including its own. Our experiments suggest a "tragedy of the commons” scenario wherein the fast growth of *V. natriegens* on the monomer GlcNAc prevents *V. 1A01* from growing, eventually limiting its ability to degrade chitin and generate monomers for both species. Thus, an indirect negative consequence of fast growth may be that it deprives the cells of the possibility to benefit from other species in environments that they cannot excel in.

P13

Towards model-informed design of antibiotic therapies:

Understanding the impact of antibiotics on bacterial physiology & growth

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Antimicrobial resistance is on the rise globally. Increased levels of resistance have been reported across bacterial strains and antibiotic compounds. In order to better target resistant bacteria, understanding the relationship between drug susceptibility and bacterial physiology in different environmental conditions is key. We present a mechanistic modelling approach to quantitatively predict antibiotic effect on bacterial growth dynamics under different environmental conditions. We focus on ribosome-targeting antibiotics, which constitute more than half of drugs used to treat infections and are among the most successful antimicrobials. We model the uptake of antibiotics and their dynamic interplay with ribosomes within an established model of bacterial growth physiology [2]. Integrating literature data on growth responses to four ribosome-targeting antibiotics (chloramphenicol, kanamycin, tetracycline and streptomycin [3]), we infer drug-associated parameters and obtain estimates that are consistent with reported literature values. Our model displays growth bistability: two possible growth states can be reached by isogenic cells experiencing the same antibiotic dose. This observation holds true for drugs that irreversibly bind the ribosomes but not for those that do it reversibly. Understanding the underlaying mechanisms of this behavior might help us gain insight on how tolerant or persistent populations of bacteria emerge.

Currently, we are working on expanding our framework to predict the levels of phenotypic heterogeneity in isogenic populations of bacteria caused by the bistable growth response. By integrating theoretical knowledge and data on growth responses, we hope to identify crucial interactions and gain further mechanistic understanding of drug action. This will bring us closer to a predictive theory of bacterial responses to antibiotics, and thus on a path to rational design of antibiotic therapy.

[1] O'Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance. Welcome Trust and HM Government; 2016.
[2] Weiße, A. Y., Oyarzún, D. A., Danos, V. & Swain, P. S. Mechanistic links between cellular trade-offs, gene expression, and growth. Proceedings of the National Academy of Sciences of the United States of America ;2015.
[3] Greulich P, Scott M, Evans MR, Allen RJ. Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics. Mol Syst Biol; 2015.

P14

Mapping microbiome taxonomy and function with Metagenome-Assembled-Genomes taxa-to-function Networks (MAGNET)*

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Thank to environmental 'omics sequencing, recent years saw a blooming of microbial ecology studies. The typical outcome of these studies is typically Metagenome-Assembled-Genomes (MAGs) and/or metagenomes, which is the collection of all the DNA from an environmental sample. Extensive effort in data aggregation, now environment-specific and extensive catalogs of MAGs are available, making it possible to not rely on huge non-representative databases, such as RefSeq. In order to describe the composition of microbial ecosystems, the standard approach is to enumerate the *taxa* or *functions* present in a community by comparing sequences to a reference database, which are not specifically designed for this purpose and are hard to integrate and compare, making mechanistic investigations and modeling difficult. At the same time, even if taxonomy and function are recognized to be non-trivially entangled [1], specific tools to asses this relation at the community level and in an environment-specific way are still lacking. Here, by extending a formerly proposed method *Core-Kaiju*, [2], we quantitatively and simultaneously describe taxonomy and function in microbial communities as MAGs-based bipartite Network (MAGNET), measuring the contribution of each MAG to each function. Being designed to work with a set of annotated MAGs, the tool can be flexibly employed by scientists willing to investigate, in principle, any system of interest. We test our tool on anaerobic digestion systems, by employing both human-gut (UHGG, [3]) and engineered digestors [4] MAGs catalog and show that the data produced allow us to quantitatively investigate, for example, functional redundancy in real-world microbial ecosystems both by mean of metagenomics and metatranscriptomics.

[1] S. Louca, M. F. Polz, F. Mazel, M. B. Albright, J. A. Huber, M. I. O'Connor, M. Ackermann, A. S. Hahn, D. S. Srivastava, S. A. Crowe, *et al.*, Function and functional redundancy in microbial systems, Nature ecology & evolution **2**, 936 (2018).

[2] A. Tovo, P. Menzel, A. Krogh, M. Cosentino Lagomarsino, and S. Suweis, Taxonomic classification method for metagenomics based on core protein families with core-kaiju, Nucleic Acids Research **48**, e93 (2020).

[3] A. Almeida, S. Nayfach, M. Boland, F. Strozzi, M. Beracochea, Z. J. Shi, K. S. Pollard, E. Sakharova, D. H. Parks, P. Hugenholtz, *et al.*, A unified catalog of 204,938 reference genomes from the human gut microbiome, Nature biotechnology **39**, 105 (2021).

[4] S. Campanaro, L. Treu, L. M. Rodriguez-R, A. Kovalovszki, R. M. Ziels, I. Maus, X. Zhu, P. G. Kougias, A. Basile, G. Luo, *et al.*, New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters, Biotechnology for biofuels **13**, 1 (2020).

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P15

Predicting drug mode of action through structural kinetic modelling framework

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Understanding how metabolism influences the immediate response to drug treatment can reveal fundamental mechanisms regarding the mode of action of drugs, their side effects, and offer fundamental insights into the adaptive mechanisms of cellular regulation. Thanks to advances in metabolomics, it has now become possible to track the dynamic changes in thousands of metabolites during drug treatment [1-2]. As a result, the challenge has moved from data generation to developing computational frameworks and tools capable of extracting functional insights from data. Kinetic modeling offers a promising avenue for exploring and understanding metabolism. This typically involves complex systems of nonlinear differential equations that require knowledge of kinetic parameters. Theoretically, kinetic models have the potential to simulate and integrate drug-induced dynamic metabolic changes. However, the practical implementation of these models faces many challenges. The primary obstacle lies in its excessively high computational demand, which in turn leads to long computation time. Additionally, a considerable number of kinetic parameters remain unknown, further limiting the applicability of this modeling approach and impeding the analysis and interpretation of modern high-throughput metabolomics data. In order to overcome these limitations, we have developed a theoretical framework based on Structural Kinetic Modeling (SKM) [3]. This uses the steady state approximation and assumes small perturbations to simplify the system of ordinary differential equations. These aforementioned approximations result in a system of linear equations, which can be easily solved. The use of linear equations allows for faster simulations, improving the scalability of the computational framework. The efficiency of the scheme is also improved by the reduced complexity of parameter space exploration, since SKM is based on the sampling of one single parameter (elasticities). By exploiting steady-state initial fluxes and metabolite concentrations, and stoichiometric information, the SKM scheme has the ability to simulate changes in metabolic processes caused by an inhibition of choice, for example the introduction of a drug-induced perturbation. The resulting dynamics obtained from several simulated perturbations can later be compared with the dynamics generated from experimental observations. Our modeling approach aims at predicting the specific location and impact of perturbations by combining simulated and experimental dynamics. As a proof-of-principle, we demonstrate the effectiveness of our approach by showing how it can integrate dynamic metabolomics data in large scale metabolic networks to predict the effects of perturbations. We achieve this by building a model of *Escherichia coli* central metabolism Ref. [4], and thereafter applying our method to extract the dynamics to individual enzyme perturbations. This was then compared to a set of “target” sources including experimental data [5], for which we show good agreement.

[1] M. Anglada-Girotto, G. Handschin, K. Ortmayr, A.I.Campos, L. Gillet, P. Manfredi, C.V. Mulholland, M. Berney, U. Jenal, P. Picotti, and M. Zampieri, Nat. Chem. Biol., **18(5)**, 482-491 (2022)
[2] S. Dubuis, K. Ortmayr, and M. Zampieri, Commun. Biol., **1(1)**, 101 (2018)
[3] R. Steuer, T. Gross, J. Selbig, and B. Blasius, Proc. Natl. Acad. Sci. U.S.A., **103(32)**, 11868-11873 (2006)
[4] E. Noor, A. Flamholz, A. Bar-Even, D. Davidi, R. Milo, and W. Liebermeister, PLoS Comput. Biol., **12(11)**, e1005167 (2016)
[5] D. Christodoulou, H. Link, T. Fuhrer, K. Kochanowski, L. Gerosa, and U. Sauer, Cell Syst., **6(5)**, 569-578 (2018)

P16

The resistance of Gram-negative bacteria to β-lactam antibiotics is primarily due to production of β-lactamase enzymes that degrade the β-lactam ring. The enzymatic degradation of these antibiotic molecules by resistant bacteria has been proposed to be a cooperative behaviour, such that populations with a higher initial density can survive at higher initial toxin concentrations. In this scenario, bacteria survive through cooperative defence due to the benefits of the enzymatic degradation of the toxin being shared between cells, so that initially denser populations would be expected to degrade toxin faster and be more likely to reach the toxin threshold before they are eliminated. To explore this scenario in detail, here, we simulate a scenario of a population of E. coli beta-lactamase producers which are partitioned in multiple subvolumes, each subvolume being filled stochastically such that the initial population size approximates the initial bacterial density. Then we simulate both deterministic and stochastic growth and death of these populations under antibiotic treatment. We show that, as expected by the cooperative beta-lactamase scenario, at high antibiotic concentrations, bacteria partitioned in many small subpopulations are able to evade antibiotic treatment. Our results show that spatial partitioning can enhance the effects of cooperative enzymatic degradation of a toxin, with potential implications for antibiotic treatment of spatially structured infections.

P17

A mathematical model to show how phage lysis promotes bacterial population growth after environmental shifts

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Microbial communities are frequently facing shifts in the environment and need to adjust their metabolism accordingly. Environmental shifts, especially from rich to poor nutrient conditions, require cells to produce a different set of enzymes to produce all cellular building blocks that they cannot obtain externally. This can lead to a dilemma, where cells lack the energy and/or building blocks to build the enzymes that would allow them to produce these in the new condition. Here, we investigate whether phage lysis can play a role during metabolic remodeling after an environmental shift by releasing cellular building blocks for the bacterial population. Experiments conducted in our group using *E. coli* as a model system show that intermediate fractions of phage lysis can indeed facilitate the growth of a population exposed to an environmental shift, overcompensating for the fraction of cells that are lysing. This positive effect is maximized for a small amount of lysing cells (~10%), highlighting a non-trivial strategy employed by communities to enhance biological fitness at the population level, while being detrimental for individual cell level. To gain insights into potential underlying mechanisms such as accelerated remodeling or benefits in maximum growth rate, we are developing a mathematical model that enables us to test various hypotheses. First findings indicate that the advantage does not come from a faster metabolic remodeling, as we previously suspected, but from a temporal increase in maximum growth rate. Overall, the observed benefit of phage lysis may represent a universal principle for coping with fluctuating environments in microbial ecology.

P18

Empirical Corroboration of *Pseudomonas fuscovaginae* Socio-Bacteriology

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Bacterial communication (often referred to as "quorum sensing") has predominantly been studied in Gram-negative bacteria [1]. Socio-bacteriology is the field of study concerned with the plethora of higher-order interactions, emerging from such bacterial communication. To reduce the immense and incomprehensible complexity of socio-bacteriological systems, and hopefully better describe, predict, and understand them, mathematical modelling is often used. However, the informative content and strength of theories depends on the degree and extent of their corroboration [2]. Here we present the quorum sensing communication system of a bacterial rice plant pathogen, *Pseudomonas fuscovaginae* [3], and showcase an array of experimental methodologies, which can generate quantitative empirical data in relation to its communication system. We hope that these experiments will be adapted to study a much wider array of bacterial species and will ultimately be used for empirical corroboration of socio-bacteriologic theories in general and further refinement of mathematical models describing these intricate and complex relationships.

[1] Suárez-Moreno, Z. R., González, J. F., Devescovi, G., & Venturi, V. (2012). Quorum sensing in gram negative bacteria: signals, role and networking. *Bacterial Regulatory Networks*, 87-122.
[2] Popper, K. (2005). *The logic of scientific discovery*. Routledge.
[3] Mattiuzzo, M., Bertani, I., Ferluga, S., Cabrio, L., Bigirimana, J., Guarnaccia, C., ... & Venturi, V. (2011). The plant pathogen Pseudomonas fuscovaginae contains two conserved quorum sensing systems involved in virulence and negatively regulated by RsaL and the novel regulator RsaM. *Environmental microbiology*, *13*(1), 145-162.

P19

Metabolic interactions with non-growing bacteria: mechanisms and ecological implications for microbial communities

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Microbial communities play a crucial role in the functioning of virtually all ecosystems, from Earth's geochemical cycles to animal health. These communities often rely on metabolic exchanges between members, termed cross-feeding, to acquire essential nutrients and factors that they cannot synthesise or derive directly from the environment. While beneficial, cross-feeding links the survival of one bacterium to the functioning of its cross-feeding partners, increasing its vulnerability. It remains unclear how the arrest of growth due to environmental fluctuations or stress impacts these interaction networks. Despite the fact that the state of non-growth represents an important aspect of bacterial life, and the predominant state in many oligotrophic ecosystems, little is known about the ecological implications of non-growing bacteria in microbial communities.

To investigate these questions, we have established a community consisting of two members of the gut microbiota: *Escherichia coli* and the probiotic lactic acid bacterium *Lactobacillus plantarum*. Using live-cell imaging of co-cultures in a microfluidic chip, we found that when switching to a minimal medium, where *L. plantarum* and *E. coli* need to cross-feed to survive, L. plantarum stops growing but is still able to maintain itself and support the growth of E. coli for over 48 hours. By integrating measurement of growth rates and information about spatial organisation, we found that this effect is only possible at short distances between the two partners. This result suggests that during environmental shifts to low-nutrient conditions, non-growing bacteria could still metabolically interact with others, increasing the functional robustness of the network. Bacteria in a non-growing state could therefore represent unrecognised actors in the organisation and function of microbial communities.

P20

Anthropogenic Influence on Microbial Community Stratification and Stability in Freshwater Sediments

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Background: Sediment microbes play a crucial role in regulating geochemical cycles and controlling greenhouse gas emissions. They often exhibit a highly ordered structure along depth profiles. This stratification not only reflects redox effects but also provides valuable insights into historical transitions, as sediments serve as important archives for tracing environmental history. The Anthropocene, a candidate geological epoch, has recently garnered significant attention. However, the human impact on sediment zonation under the cover of natural redox niches remains poorly understood. Dam construction stands as one of the most far-reaching anthropogenic modifications of aquatic ecosystems. Here we attempted to identify the ecological imprint of damming on freshwater sediment microbiome.

Results: We conducted a year-round survey on the sediment profiles of a large shallow lake in China [1]. Depth-discrete shotgun metagenomics, metataxonomics, and geophysiochemical analyses unveiled a unique prokaryotic hierarchy shaped by the interplay of redox regime and historical damming (labeled by the ^{137}Cs peak in AD 1963). Dam-induced initial differentiation was further amplified by nitrogen and methane metabolism, forming an abrupt transition governing nitrate-methane metabolic interaction and gaseous methane sequestration depth. Using a random forest algorithm, we identified damming-sensitive taxa that possess distinctive metabolic strategies, including energy-saving mechanisms, unique motility behavior, and deep-environment preferences. Moreover, null model analysis showed that damming altered microbial community assembly, from a selection-oriented deterministic process above to a more stochastic, dispersal-limited one below. Temporal investigation unveiled the rapid transition zone as an ecotone, characterized by high species richness, low community stability, and emergent stochasticity. Path analysis revealed the observed emergent stochasticity primarily came from the high metabolic flexibility, which potentially contributed to both ecological and statistical neutralities.

Conclusions: We delineate a picture in which dam-induced modifications in nutrient availability and sedimentation rates impact microbial metabolic activities and generate great changes in the community structure, assembly, and stability of the freshwater sediment microbiome. These findings reflect profound ecological and biogeochemical ramifications of human-Earth system interactions and help re-examine the mainstream views on the formation of sediment microbial stratification.

[1] Zhou, X., Lennon, J.T., Lu, X. et al. Anthropogenic activities mediate stratification and stability of microbial communities in freshwater sediments. Microbiome **11**, 191 (2023).