EXTREMOPHILES 2024
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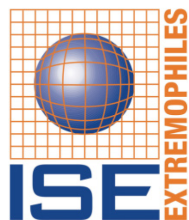
14th INTERNATIONAL CONGRESS ON EXTREMOPHILES

SEPTEMBER 22/26, 2024

ABSTRACT BOOK

PATRONAGE



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PROGRAM

Sunday 22nd September 2024	
from 16:00	Registration
	Room A (Rosa & Tulip)
18:00	**Opening remarks** **Chairperson - Konstantinos Vorgias**
18:30-19:00	**KL1** Dong-Woo Lee
19:00-19:30	**KL2** Li Huang
19:30-20:00	**KL3** Inês Cardoso Pereira
20:00	**Welcome reception**

Monday 23rd September 2024		
	Room A (Rosa & Tulip)	**Room B (Jasmin)**
09:00-10:30	**SESSION 1: Extremophiles (Panagiotis Kastritis)**	
09:00-09:30	**KL4** Tessa Quax	
09:30-10:00	**KL5** Tom Santangelo	
10:00-10:30	**KL6** Panagiotis Kastritis	
10:30-11:00	Coffee and networking	
11:00-12:00	**SESSION 2: Extremophiles (Panagiotis Kastritis)**	
11:00-11:30	**KL7** Mathias Wilmanns	
11:30-12:00	**KL8** Presentation dedicated to Mosé Rossi	
12:00-13:15	**SESSION 3a: Parallel Session 3a Extremophiles (*Vasil Gaisin + David Moreira*)**	**SESSION 3b: Parallel Session MOSE ROSSI Extremophiles and Biotechnology (*Cristina Cattò*)**
12:00	**OS1** Anna Trofimova	**OS6** Simone Antonio De Rose
12:15	**OS2** Jyothi Basapathi Raghavendra	**OS7** Cristina Cattò
12:30	**OS3** Costantino Vetriani	**OS8** Alexey Fomenkov
12:45	**OS4** Lara Vimercati	**OS9** Marika Gargano
13:00	**OS5** David Moreira	
13:15-14:30	**Lunch and networking**	
14:30-15:30	**SESSION 4a: Extremophiles (*Ok-sun Kim*)**	**SESSION 4b: Extremophiles and Biotechnology (Bjorn *Adalsteinsson + Daniela Giordano*)**
14:30	**OS10** Bledina Dede	**OS14** Naoko Okibe
14:45	**OS11** Subin Lee	**OS15** Daniela Giordano
15:00	**OS12** Christaline George	**OS16** Elizabeth Watkin
15:15	**OS13** Jiayi Jiang	**OS17** Bjorn Adalsteinsson
15:30-18:00	**Poster session with coffee and refreshments**	

18:00-19:15	**SESSION 5a: Extremophiles (*Christina Foreman)***	**SESSION 5b: Extremophiles and Biotechnology (Azadeh Zahra *Fatemi*)**
18:00	**OS18** Federica De Lise	**OS23** Andreea-Melisa Tripon
18:15	**OS19** Marco Antonio Jiménez-Santos	**OS24** Azadeh Zahra Fatemi
18:30	**OS20** Sabine Schwarzer	**OS25** Francesca Maria Pia Paragliola
18:45	**OS21** Christine Foreman	
19:00	**OS22** Matt Streets	
19:30	Free evening / meeting of ISE	

Tuesday 24th September 2024		
	Room A (Rosa & Tulip)	**Room B (Jasmin)**
09:00-10:30	**SESSION 6: Extremophiles and Biotechnology (*Nicola Curci)***	
09:00-09:30	**KL9** Beatrice Cobucci Ponzano	
09:30-10:00	**KL10** John van der Oost	
10:00-10:30	**KL11** Simon Rittmann	
10:30-11:00	Coffee and networking	
11:00-12:00	**SESSION 7: Physiology of Extremophiles (Takuro Nunoura)**	
11:00-11:30	**KL12** Purificacion Lopez-García	
11:30-12:00	**KL13** Takuro Nunoura	
	Room A (Rosa & Tulip)	**Room B (Jasmin & Lilium)**
12:00-13:15	**SESSION Session 8a Physiology of Extremophiles (*Tatsuo Kurihara + Xiuzhu Dong*)**	**SESSION 8b Extremophiles and Biotechnology (*Sara Cantera + Xu Feng*)**
12:00	**OS26** Anandi Tamby	**OS31** Sara Cantera
12:15	**OS27** Xiuzhu Dong	**OS32** Xu Feng
12:30	**OS28** Tatsuo Kurihara	**OS33** Vincenzo Zammuto
12:45	**OS29** Irene Sanchez Andrea	**OS34** Nicola Curci
13:00	**OS30** Aleksei Samolygo	
13:00-14:30	Lunch and Excursion	

Wednesday 25th September 2024		
	Room A (Rosa & Tulip)	**Room B (Jasmin & Lilium)**
09:00-10:00	**SESSION 9 System Biology of Extremophiles (*Fengping Wang*)**	
9:00	**KL14** Wolfgang Streit	

9:30	**KL15** Fengping Wang	
10:00-11:00	**SESSION 10a Physiology of Extremophiles (*Costantino Vetriani + Mohamed Jebbar Jebbar*)**	**SESSION 10b: Extremophiles and Biotechnology (Angeliki *Sitara + Angela Casillo*)**
10:00	**OS35** Masahiro Ito	**OS39** Kristy Chang
10:15	**OS36** Vasil Gaisin	**OS40** Oriana Sacco
10:30	**OS37** Mohamed Jebbar	**OS41** Angeliki Sitara
10:45	**OS38** Donato Giovannelli	**OS42** Angela Casillo
11:00-11:30	Coffee and networking	
11:30-12:45	**SESSION 11a Physiology of Extremophiles *(Heidi Smith + Javiera Norambuena)***	**SESSION 11b Extremophiles and Biotechnology (*Stefan Janecek + Onur Kirtel*)**
11:30	**OS43** Mirko Basen	**OS48** Georgios Kontellas
11:45	**OS44** Kesen Ma	**OS49** Martina Aulitto
12:00	**OS45** Heidi Smith	**OS50** Onur Kirtel
12:15	**OS46** Haruyuki Atomi	**OS51** Stefan Janecek
12:30	**OS47** Javiera Norambuena	**OS52** Maria Michela Corsaro
12:45-14:00	**Lunch and Poster session**	
14:00-15:00	**SESSION 12a: System Biology of Extremophiles (Gaël Erauso + *Khaleque Himel Nahreen*)**	**SESSION 12b: Extremophiles and Sustainable Future (Elisa *Huang Lin + Mauro Di Fenza*)**
14:00	**OS53** Himel Nahreen Khaleque	**OS57** Clemens Rausch
14:15	**OS54** Zackary Jay	**OS58** Elisa Huang Lin
14:30	**OS55** Gaël Erauso	**OS59** Mohamed A. M. Mostafa
14:45	**OS56** Francisco Issotta	**OS60** Sreemoyee Mitra
15:00-15:30	Coffee and networking	
15:30-16:30	**SESSION 13a System Biology of Extremophiles (*Manrique De La Cuba + Matteo Selci*)**	**SESSION 13b Extremophiles and Sustainable Future Future (*Miguel Paredes-Barrada + Roberta Iacono*)**
15:30	**OS61** Matteo Selci	**OS66** Miguel Paredes-Barrada
15:45	**OS62** Pamela Knoll	**OS66** Ahmad Ali Pourbabaee
16:00	**OS63** Maria Fernanda Manrique De La Cuba	**OS67** Mauro Di Fenzaa
16:15	**OS64** Memory Tekere	**OS68** Roberta Iacono
	Free evening	

Thursday 26th September 2024		
	Room A (Rosa & Tulip)	
09:00-10:00	**SESSION 14: Extremophiles and Sustainable Future (*Ondrej Uhlik*)**	
09:00-09:30	**KL16** Sung Gyun Kang	
09:30-10:00	**KL17** Ondrej Uhlik	
10:00-10:30	Coffee and networking	

10:30-11:45	**SESSION 15 Extremophiles and Sustainable Future (*Patrizia Contursi + Schlömann Michael*)**	
10:30	**OS69** Ali Shaikh Ibrahim	
10:45	**OS70** Alessia Di Fraia	
11:00	**OS71** Gabriella Fiorentino	
11:15	**OS72** Patrizia Contursi	
11:30	**OS73** Michael Schlömann	
11:45	Closing remarks	
12:00	*Poster Awards Ceremony*	
12:30	**Closing Ceremony**	

KEYNOTE PRESENTATIONS

Harnessing the extremely thermophilic bacterium *Fervidobacterium islandicum* AW-1 for a sustainable bioeconomy

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Over six billion tons of poultry feathers, classified as agro-waste, pose a significant global environmental burden due to inadequate disposal methods such as incineration and landfilling. This study aims to develop eco-friendly bioprocesses to valorize these recalcitrant feather wastes. We utilized the keratinolytic activity of the extremely thermophilic bacterium *Fervidobacterium islandicum* AW-1, isolated from Indonesian volcanic hot springs. To elucidate this unique keratinolytic activity, we performed comprehensive physiological and metabolic characterizations using multi-omics analyses. These analyses not only enhanced our understanding of keratin degradation but also facilitated the discovery of novel biocatalysts. Our findings indicate that the keratinolytic activity of this bacterium is primarily due to membrane-associated protein complexes that are highly expressed under nutrient-limited conditions, enabling direct cellular adhesion to keratin fibers. Utilizing this unique catalytic capability, we designed and developed an anaerobic fermentation process to achieve zero-waste recycling technologies. This process produces high-value products such as bioactive keratin peptides for cosmeceuticals, keratin hydrolysates for biostimulants, and hydrogen as a byproduct for biofuels. These case studies highlight the potential of extremophiles as innovative biocatalysts, paving the way for a sustainable bioeconomy and the development of future industries.

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Saccharolobus islandicus replicative primase is involved in DNA double-strand break repair

Daijiang Xiong,[1] Zhimeng Li,[2] and Li Huang[1,2]

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Archaea employ a eukaryotic-type primase consisting of the catalytic subunit PriS, the noncatalytic subunit PriL and, in some species, the second non-catalytic subunit PriX in DNA replication. It was shown that a fusion protein comprising the N-terminal domain of PriL and N-terminally truncated PriX was as efficient as PriL and PriX in facilitating primer synthesis by PriS in vitro, indicating that the highly conserved Fe-S cluster in PriL is dispensable[1]. Here we show that a mutant strain of the hyperthermophilic archaeon *Saccharolobus islandicus* encoding the fusion protein, instead of PriL and PriX, grews as well as the parent strain under optimal growth conditions but were more sensitive than the parent strain to treatment with DNA damaging agents that caused DNA double strand breaks (DSBs). Overproduction of wild-type PriL increased the tolerance of *S. Islandicus* to DNA damaging agents through enhanced error-prone DNA repair. Further evidence revealed that PriL was responsible for the repair of endogenously generated DSBs. This role of the primase is consistent with the ability of PriSL to promote annealing between DNA strands sharing microhomology and catalyze polymerization across discontinuous templates. We also show that little PriL existed during the S/G2 transition, indicating that the primase-mediated DSB repair was cell cycle dependent. Our results suggest the presence of a novel nonhomologous end joining pathway in Archaea.

Reference

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Dissimilatory sulfate reduction in archaea

Inês A. C. Pereira,[1] Ana C.C. Barbosa,[1] Sofia S. Venceslau,[1] Sinje Neukirchen,[2] Filipa L. Sousa,[2]

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Microbial dissimilatory sulfate reduction (DSR) is the main driver of the sulfur cycle and an important player in the carbon cycle in anoxic environments. Although it is well recognised that dissimilation of sulfur compounds was present on the early Earth, there is dispute regarding the onset of DSR. The capacity for DSR is quite widespread in Bacteria, in contrast to Archaea, where it has been described in limited number of hyperthermophilic organisms. Here I will discuss the evolution of DSR in archea[1,2] and our studies of enzymes and membrane complexes involved in this process, in the hyperthermophilic sulfate reducer *Archaeoglobus fulgidus*[3,4].

References

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Infection mechanisms of tailed haloarchaeal viruses

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Archaea are infected by unusual viruses that are structurally very diverse. They include viruses with unique capsid shapes such as a bottle, and viruses with cosmopolitan morphologies. An example of cosmopolitan viruses are the tailed archaeal viruses, that have a similar shape as viruses infecting bacteria. Since the archaeal cell envelope is fundamentally different from that of bacteria and archaea, archaeal viruses face different challenges when traversing the cell envelope on their way in and out of the cell. We aimed to determine if the infection mechanisms of tailed archaeal viruses are also similar to bacterial viruses. We use haloarchaea and their viruses to study viral infection mechanisms, such as viral entry and egress. In contrast to other *Haloferax* strains, *Haloferax gibbonsii* LR2-5 is very susceptible to viral infection and serves as a host for a dozen of viruses. In my talk I will present the molecular mechanisms underlying entry and egress of viruses infecting this strain. I will focus specifically on Haloferax tailed virus 1 (HFTV1), as this serves as a model for the study of tailed haloarchaeal viruses. Our results indicate that the entry and egress mechanism of this virus are quite different from bacterial tailed viruses, underlining the unique nature of archaeal viruses.

The extensive m5C epitranscriptome of Thermococcus kodakarensis is generated by a suite of RNA methyltransferases that support thermophily

Kristin A Fluke[1], Ryan T Fuchs[2], Yueh-Lin Tsai[2], Victoria Talbott[1], Liam Elkins[3], Hallie P Febvre[3], Nan Dai[2], Eric J Wolf[2], Brett W Burkhart[3], Jackson Schiltz[3], G Brett Robb[2], Ivan R Corrêa Jr[2], Thomas J Santangelo[1,3]

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RNAs are often modified to invoke new activities. While many modifications are limited in frequency, restricted to non-coding RNAs, or present only in select organisms, 5-methylcytidine (m5C) is abundant across diverse RNAs and fitness-relevant across Domains of life, but the synthesis and impacts of m5C have yet to be fully investigated. Here, we map m5C in the model hyperthermophile, Thermococcus kodakarensis. We demonstrate that m5C is ~25x more abundant in T. kodakarensis than human cells, and the m5C epitranscriptome includes ~10% of unique transcripts. T. kodakarensis rRNAs harbor tenfold more m5C compared to Eukarya or Bacteria. We identify at least five RNA m5C methyltransferases (R5CMTs), and strains deleted for individual R5CMTs lack site-specific m5C modifications that limit hyperthermophilic growth. We show that m5C is likely generated through partial redundancy in target sites among R5CMTs. The complexity of the m5C epitranscriptome in T. kodakarensis argues that m5C supports life in the extremes.

Accessing eukaryotic biology and biomolecular structure analysis using the mold *Chaetomium thermophilum* and cryo-electron microscopy

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Advances in understanding biomolecular structure and function are accelerated by thermophilic proteins – These proteins exhibit unprecedented thermostability, which is an ideal property for structural studies. Despite this, eukaryotic biology is challenging to access, as multicellularity is rarely identified in extreme environments. Here, I will present findings regarding the analysis of primary metabolic complexes from the thermophilic mold *Chaetomium thermophilum* (also *Thermochaetoides thermophila DSM 1495*) as a model system. *C. thermophilum* is notable for being a eukaryote with a high temperature tolerance (60 °C) while its optimal growth temperature is 50–55 °C[1]. By employing an efficient biochemical protocol to retrieve endogenous protein complexes from its native cell extracts[2], we have resolved multiple protein complexes and their higher-order assemblies involved in primary metabolism utilizing cryo-electron microscopy and computational modeling. Specifically, we have resolved integrative structures of transient megadalton complexes[3] involved in pyruvate oxidation[4,5], succinyl-coA production[6], fatty acid synthesis[7], and inositol biosynthesis. The mechanistic insights provided by decoding these higher-order assembly architectures, dubbed as *metabolons*[8], unveil disorder-to-order transitions and flexible, long-range interactions, during substrate shuttling and catalysis. I expect that our model organism and associated methods are transferrable to other systems, and provide access to hundreds of protein complexes in order to be studied and resolved in their endogenous states, promoting *in situ* structural biology[9]. Such perspective will have direct implications in advancing applications in medicine and biotechnology, as well as in disseminating basic principles of eukaryotic metabolism.

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Discovery of unexpected orphan enzyme function from extremophilic organisms using high-resolution structural biology

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The emergence of life under most diverse conditions remains one of the most fascinating areas in research to advance our knowledge of the origins of life, the adaptation of living organisms to extreme environmental conditions, and to facilitate the assessment of future prospects for life under rapidly changing climate conditions. Extreme conditions have consequences at all levels of the organization of living organisms, up to the molecular and atomic level on how biological structures are organized. This presentation will provide an overview of the current state of the art on the study of organisms from extremophilic environments from the perspective of molecular and even atomic adaptations to such conditions. This will include recent research highlights from our group, using previous data from the genome of the thermophilic anaerobic bacterium *Thermoanaerobacter thermohydrosulfuricus*[1]. This bacterium was originally isolated from the Solar Lake located on the Sinai peninsula, which has an extreme marine environment with a temperature range from 16 to 60 °C and high level of salinity[2]. We have recently focused on the structural and functional elucidation of an orphan enzyme of this bacterium[3]. A high-resolution structure in the presence of an unexpected endogenous C18 monoacylglycerol ester reaction intermediate from the expression host allowed its functional characterization as a prototypic long-chain monoacylglycerol lipase. Knowledge of the molecular details of the substrate binding site allowed us to modulate the enzymatic activity by the adjusting protein/substrate interactions, demonstrating the potential of our findings for future biotechnological applications.

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Flexibility and regulation of genetic code decoding via translational recoding in archaea

Federica De Lise,[1] Laura Kuschmierz,[2] Oriana Sacco,[1,3] Alberto Bobbio,[1,3] Andrea Strazzulli,[3,4] Bettina Siebers,[2] Marco Moracci,[3,4] and Beatrice Cobucci-Ponzano,[1]

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The genetic code and its translation, once considered to be universal and immutable, are now understood to be flexible. Translational recoding, where ribosomes deviate from standard translational rules in a programmed manner, includes various events during gene translation, such as stop codon readthrough and programmed ±1 frameshifting. Often discovered by chance, these events regulate protein expression at the translational level and are well-characterized in viruses, bacteria, and eukaryotes. This flexibility in genetic code decoding is suggested to be an evolutionary trait that benefits microorganisms under certain physiological conditions, increasing their fitness. In Archaea, translational recoding has been demonstrated in the decoding of the 21st and 22nd amino acids, selenocysteine and pyrrolysine, respectively. Only one case of programmed –1 frameshifting has been reported, and understanding the molecular mechanisms regulating it has only recently started to emerge. Potential genes whose expression could be regulated by translational recoding have been hypothesized in other archaeal species. However, despite the significant implications these recoding events could have for the physiology and adaptation of life in extreme environments, their characterization lags behind. These findings highlight the urgent need for further research in this field.

Reference

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Thermophilic nucleases – from biology to biotechnology

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Just like mesophilic microbes, also thermophilic bacteria and archaea use a wide range of immune systems to defend themselves against invasions by viruses. The DNA-guided Argonaute nuclease is just one example of the huge arsenal of innate defence systems. On top of that, an adaptive immune system (CRISPR-Cas) has been discovered, different variants of which are present in about half of the currently known prokaryotes. Several RNA-guided CRISPR-Cas systems have successfully been repurposed, not only as versatile genome editing tools, but also for diagnostics applications. The discovery of new Argonaute variants as well as new CRISPR-associated systems continues, revealing novel biology and potential for development of powerful applications. During this lecture some recent examples will be described.

CO_2-fixation by a thermophilic archaeon for proteinogenic amino acid production

Simon K.-M. R. Rittmann

The biotechnological production of proteinogenic amino acids is a cornerstone of global food production and security. Proteinogenic amino acids are the building blocks of proteins. Methanogenic archaea are known to excrete all proteinogenic amino acids into the culture supernatant. *Methanothermobacter marburgensis* is one of the suitable organisms for proteinogenic amino acid production. Different patterns of amino acids can be secreted by *M. marburgensis* into the supernatant according to specific process conditions. The bioprocess is operated in fed-batch or continuous culture mode and the carbon substrate for amino acid production is CO_2. Proteinogenic amino acid production from CO_2 could change the amino acid production technology on a global scale.

Adaptations and metabolic flexibility of diverse archaea thriving in geothermal chaotropic brines near life limits

Purificacion López-García, Ana Gutiérrez-Preciado, Bledina Dede & David Moreira

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Few described archaeal, and fewer bacterial, lineages optimally thrive at salt-saturating conditions, such as solar saltern crystallizers (salinity above 30%-w/v). They accumulate molar K^+ cytoplasmic concentrations to maintain osmotic balance ('salt-in' strategy). Compared to NaCl-saturating solar salterns, many athalassohaline hypersaline ecosystems are enriched in macromolecule-disorganizing, chaotropic salts, e.g. Ca-, Mg-, Fe- or Li-salts, and display other physicochemical extreme conditions. How do organisms adapt to these polyextreme conditions and what is the permissive limit for life? To answer, we studied a series of geothermally influenced hypersaline ecosystems with increasing chaotropicity in the north Danakil Depression (Ethiopia). While several of these highly chaotropic systems seem devoid of life, 16S rRNA gene metabarcoding and metagenomic analyses showed that the most polyextreme environments where life was detected, microbial communities were overwhelmingly dominated by extremely halophilic archaea (up to 99% of the total community). Inferred proteomes from Danakil metagenomes and metagenome-assembled genomes (MAGs) compared to those of freshwater, seawater and solar salterns of increasing salinity, showed that some of these archaea encode the most acidic proteomes ever observed. Despite phylum-level diversity decreasing with increasing salinity-chaotropicity, and unlike in solar salterns, adapted archaea were extremely diversified in Danakil ecosystems, challenging the general idea that diversity decreases as extreme conditions increase. Several of these archaeal lineages correspond to new families. Metabolic flexibility to utilize multiple energy and carbon resources generated by local hydrothermalism along feast-and-famine strategies seemingly shape microbial diversity in these ecosystems near life limits.

Identification of novel reactions in well-known metabolic pathways using a high-resolution MS system

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The central carbon metabolic pathways and amino acid biosynthetic pathways are clues to understand the microbial physiology. However, the metabolic pathways estimated by genome sequences always include missing links. In such cases, tracer-based metabolomics is a powerful tool to figure out the most probable metabolic pathways, but tracer-based metabolomics is not applicable of in most of the cases because the conventional metabolomics require amount of biomass that are difficult to obtain for environmental microbes. A microfluidic capillary electrophoresis-mass spectrometry (CE-MS) method using Orbitrap Fusion MS is a rapid and highly accurate method to determine isotopomer patterns in isotopically-labelled compounds. Currently, we have developed a novel method for tracer-based metabolomics using CE-MS for underivatized amino acids. The novel method consisting of a ZipChip CE system and a high-resolution Orbitrap Fusion MS allows us to obtain highly accurate data from 1 µL of 100 nmol/L mol proteinogenic amino acids comparable to 1 x 10^4-10^5 prokaryotic cells. This means that we can examine the central carbon metabolic pathways and amino acid biosynthetic pathways from cells grown in few ml medium [1]. In addition, we also applied the combination of HPLC and the high-resolution MS to identify cellular organic acids [2]. Here, we will introduce updates of ^{13}C tracer-based metabolomics using the Orbitrap Fusion MS.

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Plastic pollution, a global challenge: Extremozymes as useful tools for plastic recycling

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Petroleum-based plastics are durable and accumulate in all ecological niches. Knowledge on enzymatic degradation is still very limited[1]. Today, less than 200 verified plastics-active enzymes are available in the PAZy (www.pazy.eu) data base. During my presentation I will give an overview on the current status and potential of plastic active-enzymes and microorganisms for plastic recycling and remediation. The best studied enzymes are those acting on the polymers polyethylene terephthalate (PET) and polyurethane (PUR, ester-based). While in general promiscuous and mesophilic hydrolases are able to turn over PET at very low rates, the highest catalytic efficiency is affiliated with enzymes that work at temperatures above 65°C. To advance this field we have developed and applied a highly efficient Hidden-Markov-Model based search pipeline for the identification of novel hydrolases acting on ester-based polymers. Using this pipeline in combination with in vitro transcription and translation approaches, we were able to enrich the known biodiversity of PETases and PURases significantly by mining in global metagenomes. Within my presentation I will highlight advances in metagenome screening technologies for plastic-active enzymes. I will further highlight our work on an archaeal promiscuous feruloyl esterase exhibiting degradation activity on semi-crystalline PET[2]. The enzyme was found by a sequence-based metagenome search, and it is derived from a non-cultivated, deep-sea Candidatus Bathyarchaeota archaeon. Biochemical characterization demonstrated that PET46 is a promiscuous, heat-adapted hydrolase. Its crystal structure was solved at a resolution of 1.71 Å. It shares the core alpha/beta-hydrolase fold with bacterial PETases, but contains a unique lid common in feruloyl esterases, which is involved in substrate binding.

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MCT-S : Microbially mediated Carbon Transformation in marine Sediments

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Microbes play essential roles in Earth's elemental cycling, significantly influence Earth's climate, particularly through production and consumption of potent Greenhouse gases—carbon dioxide, methane, and nitrous oxide. Marine sediments, one of the largest microbial habitats, harbours the largest organic carbon reservoir on Earth in which microbial transformation is considered key process controlling the carbon flux. In this talk, I will introduce a conceptual model MCT-S: Microbially mediated Carbon Transformation in marine Sediments. This model aims to enhance our understanding on the roles of microbes play in carbon cycling within marine sediments. I'll report our recent investigations on sedimentary archaea using a combination of geochemistry, OMICS, modelling, and cultivation approaches, with a particular focus on the mechanisms and potential ecological effects on recalcitrant organic carbon degradation, methane production and consumption.

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Biohydrogen production from industrial by-product gas using a hyperthermophilic archaeon, *Thermococcus onnurineus* NA1

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Hydrogen (H2) is attracting widespread attention as a promising energy carrier, which is primarily produced from fossil fuels. Biological hydrogen (biohydrogen) production is a promising alternative since it has potential to considerably reduce cost and environmental impact by harnessing renewable resources. We have been studying about biohydrogen production in *Thermococcus onnurineus* NA1 isolated from a deep-sea hydrothermal vent as a biohydrogen producer. The genome of *T. onnurineus* NA1 encodes seven different [NiFe]-hydrogenases such as cytoplasmic hydrogenases (SulfI SulfII and FrhAGB-encoded hydrogenase) and membrane-bound hydrogenases (Mbh, Mch, Mfh1 and Mfh2) [1,2,3]. Particularly, Mch was identified to play key roles on hydrogen production and energy conservation in CO utilization. Multi-omics analysis allowed us to understand how the hydrogenase gene was differentially expressed in the presence of CO and essential regulators such as corQR[4,5] and TON_1525[6,7] were identified. By manipulating those regulators or employing metabolic engineering, biohydrogen productivity was significantly enhanced in *T. onnurineus* NA1 and the optimization of continuous biohydrogen production was carried out. To make biohydrogen production cost-effective, we attempted to utilize industrial waste gas as a cheap feedstock such as byproduct gas from steel-mill process or syngas from waste[4,8,9]. The current status of biohydrogen production from industrial by-product gas will be discussed.

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Phylogenetic novelty of prokaryotes in subsurface thermal waters of cultural heritage significance

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Many subsurface waters are characterized by extreme, yet stable and unique environments that have allowed their indigenous microorganisms to evolve over millennia. The microorganisms found in such waters are phylogenetically and ecologically unique. Western Bohemia is rich in thermal springs, the origin of which is related to volcanic activity associated with the folding of the Alps. The most famous and important from a cultural and hydrogeological point of view is the Vřídlo spring in the famous spa town of Karlovy Vary, whose water is approximately 35,000 years old when it reaches the surface. The spring structure of Karlovy Vary is anchored at a depth of several kilometers, where the resulting mineral water is formed. A much rarer source of thermal water is the combination of geothermal activity with the decay of radioactive elements. One of the few places with radon-saturated water is the town of Jáchymov, also in western Bohemia. This paper presents data from culturomic and metagenomic analyses of prokaryotes from Jáchymov and Karlovy Vary. Culturomics[1, 2] led to the isolation of a number of new species, and several other strains were isolated that can be considered more extreme than some model bacteria, e.g. *Deinococcus radiodurans*. At the same time, the enormous microbial diversity of these unique ecosystems was revealed, far exceeding expectations in terms of both phylogenetic and functional/metabolic novelty.
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ORAL PRESENTATIONS

Novel *Thermus* bacteriophages encode CRISPR arrays involved in conflict with mobile genetic elements

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Thermus thermophilus is a widespread hyperthermophilic bacterium inhabiting terrestrial hot springs. Bacteria of this genus are valuable model organisms and an important source of thermostable enzymes. Bacteriophages infecting *Thermus* are sparsely studied albeit these phages are of interest both because of their unusual developmental strategies and novel enzymes they encode. In this work, we isolated phages infecting *T. thermophilus* from hot springs located at Kunashir (Kuril Islands, Russia) and Nokalakevi (Georgia) and sequenced their genomes. Genomes of some isolated bacteriophages display are highly similar to previously described *Thermus* phages P23-45, phiMa, IN93, and phiOH3. However, the genomes of two highly similar phages, Lalka8 and Lalka27, isolated, respectively, in Kunashir and Nokalakevi, display very limited similarity with known viral genomes and represent a distinct novel group of *Thermus* viruses.

Genomic assemblies of Lalka8 and Lalka27 phages comprise 72,376 and 69,092 kbps and encode 89 and 85 predicted open reading frames. Lalka8 encodes an integrase followed with a sequence matching the 3' segment of alanine tRNA suggesting that this phage is capable to integrate into the host genome using the tRNA gene as attachment sites. Both phages encode proteins involved in evasion of host restriction-modification systems, including orphan DNA methyltransferases and DarB/Ulx proteins. Strikingly, Lalka8 and Lalka27 encode CRISPR arrays with identical repeats separated by unique spacers. A similar CRISPR-containing locus was discovered in metagenomic sequences from compost (**Figure 1**). Some spacers match sequences of integrative conjugative elements (ICE) of *Thermus* suggesting that there is a competition between these phages and ICEs (**Figure 2**). The repeat sequences of phage-encoded CRISPR arrays do not resemble the repeats of previously characterized CRISPR arrays of *Thermus* or other organisms. The Lalka phage CRISPR arrays are located immediately downstream of *tnpB* genes. Given that TnpB proteins are closely related to Type V CRISPR-Cas effector proteins we propose that phage-encoded *tnpB*-CRISPR loci may represent a new variant of Type V CRISPR-Cas systems.

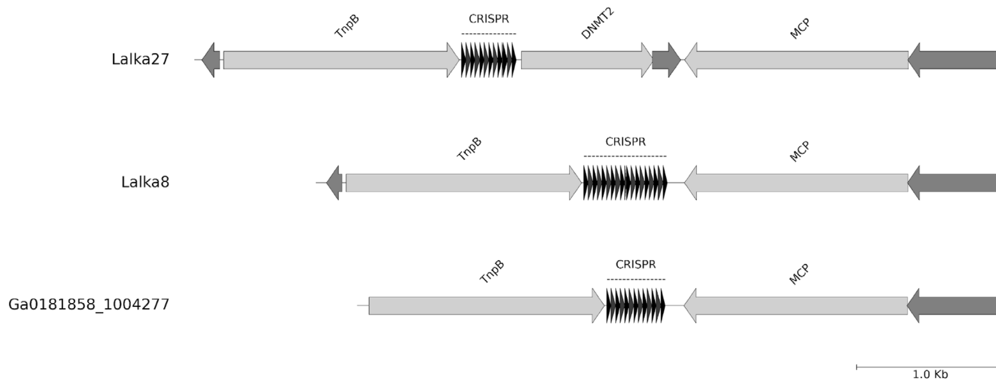

Figure 1. CRISPR loci of Lalka27 and Lalka8 phages and of a metagenomic contig. Abbreviations: DNMT2 – DNA methyltransferase; MCP – major capsid protein.

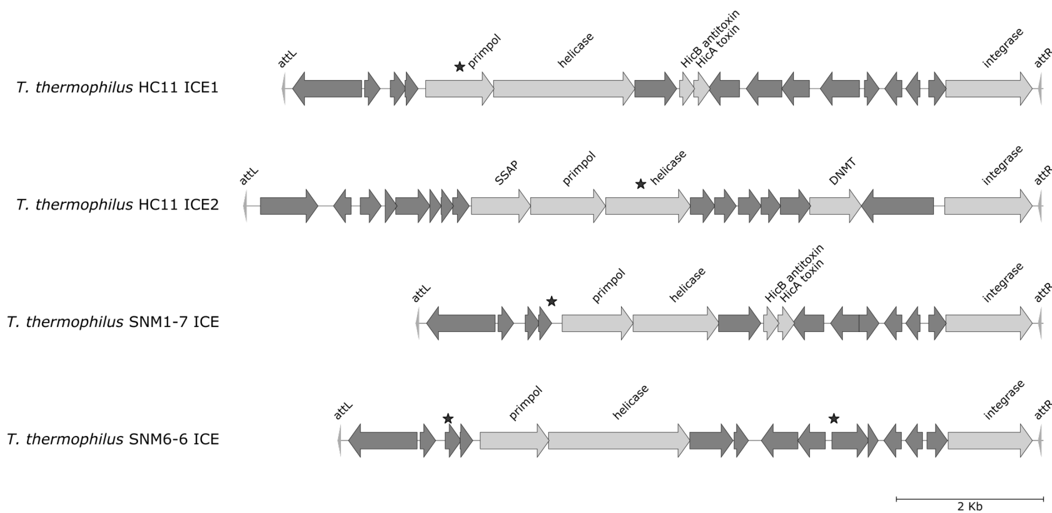

Figure 2. Spacers derived from phage-encoded CRISPR arrays matching *T. thermophilus* mobile genetic elements. The locations of spacer-matching sequences are shown with stars. Abbreviations: attL/attR – left and right junctions of host genome and ICE; primpol - primase-polymerase; SSAP - single-strand DNA annealing protein; DNMT – DNA methyltransferase.

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Assessing the lower limits of life detection in extreme environments at the picogram level using nanopore technology

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The biosphere of Earth extends over the lithosphere, hydrosphere, cryosphere, and atmosphere. Some of these habitats have been poorly characterised, partly because of the low concentration of cells, and partly because of the technical difficulties in studying some of these ecosystems. The lowest detection limit of active life forms is a topic of interest for the study of biodiversity on Earth and the possible detection of life in the rocks of Mars (Astrobiology). Deoxyribonucleic acid (DNA) being one of the incontrovertible biosignatures is an easy target, however due to its low abundance in certain environments, one of the biggest challenges is the ability to extract it efficiently and characterise it without any amplification. In this work, we describe a method for characterising low biomass habitats in all three states of environments: bioaerosols (gaseous state), MMS-2 Martian soil simulant (solid state) and brine pool (liquid state) without DNA amplification. Our study with *E. coli* and *S. cerevisiae* DNA samples showed that the MinION sequencer (Oxford Nanopore Technologies) can unequivocally detect and characterise microbes with as little as 2 pg of input with just 50 active nanopores. This value is also at the level of background contamination associated with the reagents, filters, ambient, and so forth. Furthermore, cultivating the natural MMS-2 Mars regolith simulant exposed to just atmospheric water vapour showed one way to characterise the microbiome of any terrestrial rock sample or Mars samples without additional nutrients or water. After seven days of incubation, the regolith samples showed an increase in biomass, accounting for 600 to 800 pg of extractable DNA. We demonstrate that an adapted sampling and extraction protocol, together with the analysis of the DNA sequenced using MinION sequencer allows for exploring the microbial taxonomic composition of habitats poorly described to date.

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The chemosynthetic microbiome of deep-sea hydrothermal vents across space and time

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Microbial biofilms colonize mineral and biological substrates exposed to fluid circulation at deep-sea hydrothermal vents, providing a biologically active interface along redox boundaries. Since many biofilms at deep-sea vents are associated with invertebrates, microbial distribution and abundance are not only constrained by local fluid geochemistry, but also through host-microbe interactions. In this study, we examined the spatial distribution and diversity of established microbial biofilm communities collected from three distinct biological regimes characteristic of the East Pacific Rise (9°50 N, 104°17 W) vent system, as well as newly established biofilms. 16S rRNA-based amplicon sequencing revealed that Campylobacterota of the *Sulfurimonas* and *Sulfurovum* genera dominated newly-formed biofilms across all biological regimes. Statistical analyses using fluid chemistry data from each sampling site suggest that community composition is significantly impacted by biofilm age, temperature and sulfide concentration, and to a lesser extent, locality. Further, metatranscriptomic analyses were used to investigate changes in community gene expression between seafloor and subseafloor biofilms. Our findings revealed differences in the type and abundance of transcripts related to respiratory pathways, carbon fixation, and reactive oxygen species (ROS) detoxification. Overall, this study provides a novel conceptual framework for evaluating biofilm structure and function at deep-sea vents by showing a transition from a niche-specific pioneer microbial community in newly-formed biofilms, to a complex population of increased diversity in established biofilms and by identifying key changes in gene expression in taxonomically similar biofilms during the transition from the shallow subseafloor to the seafloor.

Unique Antarctic stromatolites reveal diverse taxa and metabolic complexity: implications for the early evolution of life

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Lake Untersee is a perennially ice-covered lake located in Queen Maud Land, Antarctica. This habitat serves as an ecological analog of early Earth and other worlds in our solar system characterized by thick permanent ice covers, such as Enceladus and Europa and even ancient ice-covered lakes on Mars[1,2]. This unique environment supports a vast system of mat morphologies: pinnacles, flat mats and modern, large conical stromatolites. While pinnacles and flat mats are common throughout other Antarctic ecosystems, large complex cones are the first report of such structures in a modern environment[3]. These stromatolites consist of a top pigmented layer of photosynthetic Cyanobacteria covering a soft structure of alternating laminations of sediment and microbial organic matter[3,4]. These modern cone structures are extremely intriguing as Lake Untersee is the only environment on Earth where they have been found to date. Initial studies utilizing 16S and 18S rRNA gene sequencing and light microscopy have provided insights into the microbial composition of these structures[4,5,6], but the mechanisms driving their formation remain elusive. We reconstructed metagenome-assembled genomes (MAGs) from bacterial phyla inhabiting pinnacle and cones that grow adjacently under the same conditions, including many rarely observed phyla. Microbial structures are inhabited by distinct communities with an abundant cyanobacterium *Microcoleus* defining cone-shaped structures and *Elainellacea* increasing in pinnacle-shaped structures suggesting cyanobacteria influence mat morphologies[7]. We identified sharp partitioning from upper to lower mat layers in community composition and metabolic potential including abundance of photosynthetic pathways in the upper light-receiving laminae of the mat, and heterotrophic pathways in the lower layer. Metagenomic analysis shows that Lake Untersee is the first Antarctic Lake with substantial ammonia oxidising Nitrospiracea and *amoA* genes in benthic microbial mats. Genomic capacity for recycling is prevalent across MAGs, highlighting the importance of nutrient scavenging in ultra-oligotrophic environments. The next step we are working on is the generation of metatranscriptomes of several layers of conical stromatolites, which will allow to identify which microbial components are active and which genes of the community are expressed.

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Multiple independent adaptations of archaea to hypersaline environments

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Four groups of archaea (Halobacteria, Nanohaloarchaeota, Methanonatronarchaeia and Halarchaeoplasmatales) have adapted to thrive in saturating salt concentrations (>30%). These archaea maintain osmotic equilibrium with their hypersaline environment by accumulating molar concentrations of intracellular K^+, which has led to the acidification of their proteomes to avoid protein aggregation. The evolutionary history of these adaptations enabling high salt tolerance remains poorly understood, in particular because the phylogenetic position of several lineages is conflicting. To address this question, we have improved the taxon sampling of extremely halophilic archaea and applied state-of-the-art phylogenetic approaches designed to cope with the strong compositional biases of their proteomes[1]. We have characterized two new uncultured lineages, Afararchaeaceae and Asbonarchaeaceae, which break the long branches at the base of Halobacteria and Nanohaloarchaeota, respectively. We have obtained 13 metagenome-assembled genomes (MAGs) of these archaea from metagenomes of hypersaline aquatic systems of the Danakil Depression (Ethiopia). Our phylogenomic analyses including these taxa and a large collection of conserved protein markers allowed us to obtain a well resolved phylogeny of extremely halophilic archaea. It shows that at least four independent adaptations to extreme halophily occurred during archaeal evolution. Gene-tree/species-tree reconciliation analyses shows that gene duplication and horizontal gene transfer have played an important role in this process, for example, by spreading key genes (such as those encoding K^+ transporters) across extremely halophilic lineages.

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Rapid discovery and development of enzymes for novel and greener consumer products (RadicalZ)

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The need to transition from non-renewable resources for chemical production to more sustainable alternatives is evident. Enzymes present a green solution to this, replacing petroleum-based chemistry in industrial operations. RadicalZ is dedicated to accelerating the process of enzyme discovery and development, a current impediment to the implementation of a biocatalysis drive circular economy. The urgent requirement for enzymes capable of degrading synthetic plastics like polyethylene terephthalate (PET) to mitigate microplastic pollution is clear. Yet, the absence of efficient screening methods for potential enzyme candidates poses a challenge. RadicalZ is designed to overcome this hurdle by expediting enzyme discovery and development through ultrahigh-throughput screening techniques. These techniques involve the evaluation of enzyme libraries, compartmentalized in water-in-oil emulsion droplets, for the necessary activity.

We present a method for the ultrahigh-throughput microfluidic screening of evolved thermophilic enzymes utilizing fluorescence activated droplet sorting. We have chosen fluorescein dibenzoate (FDBz) as the fluorogenic probe for this process. FDBz, with its PET-like ester bonds connected to a benzene group, can be hydrolyzed by PETases to produce fluorescein monobenzoate[1]. As FDBz is not a reactive substrate of common esterases, it results in a low fluorescence background in cell lysates. The method involves four stages: 1) The creation of a mutant library through error-prone PCR. 2) The incubation of droplets that encapsulate individual cells, a lysis agent, and FDBz. 3) The screening and sorting of droplets to identify enhanced PET-degrading enzymes. 4) The sequencing of the evolved enzyme and additional evolution cycles to boost activity. *Thermogutta terrifontis* esterase 2 (TtEST2)[2], is utilized as a benchmark to set up the micro-fluidic experiments. Unlike most enzymes from the α/β-hydrolase family 3, TtEST2 lacks a significant portion of the 'cap' domain, and its active site cavity is open to the solvent, enabling it to accept larger substrates like PET. TtEST2's activity against FDBz is a promising sign of its ability to digest PET, and efforts are underway to enhance its activity towards larger substrates.

Moreover, other potential PETase sequences have been discovered in the HotZyme Exeter DNA database, which houses novel thermophilic genomes and metagenomes, as well as other public databases. These sequences have also demonstrated activity against FDBz. This research lays the groundwork for assessing PETase activity using PET film and fibers, with the aim of developing label-free microfluidic screening methods for the directed evolution of these industrially significant enzymes.

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Bio-cleaning of graffiti on urban surfaces using extremophilic microorganisms inhabiting spray paints

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Every new graffiti on urban surface provides a novel ecological niche for microorganisms. These graffiti-based ecosystems are inhospitable and extreme environments. Spray paint contains xenobiotic synthetic organic substances both as binders and pigments as well as heavy metals. Additionally, they are nutrient-poor habitats, subjected to rapid changes in temperature and exposed to intense solar radiation, wind and desiccation. These extreme conditions promote the grow of highly specialized communities of microorganisms living in the mode of biofilm.

In the last decades, the idea to employ these highly adapted microorganisms in new biotechnological microbial methodologies has gradually increased. Millions of urban and heritage surfaces in most cities worldwide are defaced by graffiti vandalism, resulting in economic losses of millions of euros a year. Unfortunately, current cleaning protocols are not often completely effective. Avant-garde studies have shown that microorganisms offer a powerful, low-cost, safety and eco-friendly alternative method to remove graffiti from urban surfaces. However, a ready-to-use biocleaning procedure for spray paints is lacking.

In this research a selection of extremophilic microorganisms to remove graffiti from surfaces has been performed. Isolation has been carried out directly from the extremophilic community naturally inhabiting graffiti surfaces, already adapted to the extreme environmental conditions provided by the spray paint.

Graffiti samples were prepared with silver and black spray paints. The indigenous microbial community inhabiting the paint was forced to use the paint was the sole source of energy and carbon. At the beginning of the experiment and after 2, 5, 7 and 14 days, the microbial grow was monitored by plate count assay and cellular activity was evaluated by total protein quantification. At three time steps, the alive biomass was recovered, the DNA was extracted and the microbial community was identify by Illumina MiSeqDNA sequencing. Results highlighted that after 14 days, a community of cultivable, alive and active cells was still present on the painted surface. Illumina analysis indicated that these communities were mostly composed by two bacteria of the Enterobacteriaceae family; no fungi were isolated.

Complete Genome Assembly and Methylome Dissection of two Archaeal species *Methanococcus aeolicus* PL15/H^p and *Methanobacterium wolfei* DSM 2970

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Although restriction-modification systems are found in both Eubacterial and Archaeal kingdoms, comparatively less is known about patterns of DNA methylation and genome defense systems in archaea. Two Archaeal species, *Methanococcus aeolicus* PL15/H^p, a strain of the CO_2-reducing methanogenic archaeon and a commercial source for MaeI, MaeII and MaeIII restriction endonucleases [1] and *Methanobacterium wolfei* DSM 2970, the original source of the MwoI restriction endonuclease [2] were previously isolated, characterized and deposited in the German collection of archaea [3]. However, the complete genome and methylome information for these two species was not available. The next generation sequencing platforms such as Single Molecule Real Time (SMRT), allow not only the sequence and assembly of genomes [4], but also enable the determination of epigenetic modification patterns [5-7]. The combination of bioinformatics together with cloning and expression of candidate genes and MS-MS analysis confirmed all of the methyltransferase genes responsible for their modified motifs. The *M. aeolicus* PL15/H^p genome consists of a 1.68 megabase circular chromosome predicted to contain 1,615 protein coding genes and 38 tRNAs. The sequencing of *M. wolfei* DSM 2970 also revealed a small genome of 1.7 megabases with a circular chromosome, predicted to contain 1,817 protein coding genes and 39 tRNAs. In addition, a combination of SMRT sequencing, homology-based genome annotation, and recombinant gene expression identified five restriction-modification (RM) systems encoded by *M. aeolicus* [8], including the methyltransferases and site-specific endonucleases of MaeI, MaeII, MaeIII and two RM systems including the methyltransferase and site-specific endonuclease, MwoI, for *M. wolfei* DSM 2970.We report the complete closed genomes of *Methanococcus aeolicus* PL15/H^p and *Methanobacterium wolfei* DSM 2970. The sequencing data are publicly available from NCBI. In addition, the complete methylome of these two strains and the identified RM systems may help other investigators to develop novel genetic systems and create efficient transformation protocols for these archaeal species.

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Extreme environments for extreme environmental challenges: exploration of a novel thermophilic pet hydrolyzing enzyme

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The use of plastics has exponentially spread since the last decades of 1900s, due to their low production costs, versatility and durability. This latter feature, combined with their excessive consumption, caused a rapid accumulation of plastics and devastating environmental effects[1]. Among plastics, polyethylene terephthalate (PET) is one of the most daily used, finding applications in the manufacture of single-use disposable products and accounting around 6.2% of plastics production[2]. Likewise other plastic materials, the end of life of PET products can go towards recycling, incineration, landfill storage or environmental release; however, during the last decade a class of enzymes able to hydrolyse PET (PHE) was discovered, leading to environmentally sustainable treatments of PET waste[3,4].

In this scenario, a novel thermophilic enzyme, called PP_EG, was identified from geothermal metagenomic samples. It was biochemically characterized and its ability to hydrolyse PET, into terephthalic acid (TPA), along with mono(2-hydroxyethyl)-TPA (MHET) and bis(2-hydroxyethyl)-TPA (BHET) units, was proved. Additionally, the thermostability of PP_EG pushed the depolymerisation at higher temperature than mesophilic enzymes, increasing the mobility of PET chains and, therefore, facilitating their hydrolysis[5]. This study offers the possibility to expand the information regarding PHEs, which are still limited according to the Plastics-Active Enzymes Database (PAZy – www.pazy.eu) and opens new perspectives on PET up-cycling.

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High microbial metabolic versatility in geothermally influenced lakes across a salinity gradient in Danakil Depression

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Lacustrine environments in active tectonic and volcanic regions commonly exhibit unique physico-chemical conditions such as high temperature, salinity, and pH variations associated with input from geothermal groundwaters. These extreme environments harbor diverse microbial communities, contributing to local biogeochemical cycles, as well as offering insights into Earth's evolution. Yet, these microorganisms remain largely unexplored. Here, we analyzed the microbial community in nine previously undescribed and unnamed lakes of the Danakil Depression, a unique and extreme environment created by the divergence of the Arabian, African, and Somalian tectonic plates. The studied lakes, of diverse hydrochemistry, span a salinity gradient from around half seawater values to saturation. They are geothermally active, and feature neutral pH levels with temperatures around 30°C. By combining 16S and 18S rRNA gene metabarcoding analyses, metagenomics, statistical and geochemical analyses, we describe the lakes and their microbial communities, as well as their predicted metabolic functions along this gradient of extreme conditions. We uncover a diverse bacterial community consisting of Cyanobacteriota, Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Verrucomicrobiota, with an increase in archaeal abundance in hypersaline lakes. Our analysis highlights a notable shift in photoautotrophic clades along the salinity gradient. The predominant Cyanobacteria in saline lakes (1.1 – 6%) belongs to a new genus in Thermostichales and exhibits the capability for sulfur oxidation, partial denitrification, and hydrogen production. On the contrary, the predominant Cyanobacteria in hypersaline lakes (6 – ≥~20%) are *Halothece salina* and a novel species of Halothece. These species harbor genes for sulfur oxidation and partial denitrification, enabling them to perform photosynthesis under both oxic and anoxic conditions. Moreover, we observed an increase in sulfur oxidation and denitrification genes in hypersaline lakes, along with previously undescribed clades associated with Chitinophagales, Balneolales, and Opitutales, some capable of partial denitrification and sulfur oxidation. The investigation of these lakes highlights the high metabolic versatility and discovery of new taxa, providing further insights into the diverse adaptation and resilience of microorganisms in increasingly extreme environments.

A method evaluation for analyzing microbial communities in ice cores of Styx Glacier, Antarctica

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Glaciers, despite holding over 70% of the Earth's freshwater, have remained largely unexplored by microbiologists due to difficulties in accessing glaciers, low microbial biomass, and high contamination risks. To understand microbial communities of glaciers, which require unconventional methods unlike general environmental microbial community analyses, we evaluated four distinct approaches to identify the most effective method: 1) Direct 16S rRNA gene-based PCR amplification of raw samples 2) Nested PCR with raw samples using various combinations of primers 3) PCR after bead-based DNA extraction from concentrated cells using FACS (fluorescence-activated cell sorting) 4) Manual DNA extraction after FACS-based cell concentration, followed by PCR amplification. Following a thorough evaluation, we identified method 4 as the most effective approach for our samples. After FACS-based cell concentration, DNA was manually extracted using the Phenol-Chloroform method. High-fidelity PCR amplification was then performed to obtain the final PCR products. We analyzed the microbial communities of Styx Glacier, Antarctica, using this method. We anticipate this method could be a valuable tool to analyze communities of glacial microbiomes enduring the harsh conditions of extreme low temperature, oligotrophy, and desiccation.

Biogeography of photosynthetic microbial biofilms in the hot springs of southeast asia

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Hot springs are tractable model systems in microbial ecology for investigating the interactions of photosynthetic microbial biofilms. This is because they occur across broad geographic scales, possess readily identified major abiotic variables, and are subject to minimal influence from metazoans. Despite this regional scale investigations are lacking, and major questions persist concerning the evolutionary drivers responsible for biofilm turnover at broad geographic scales. Here, we present the largest study to date, incorporating concurrent measurement of biotic and abiotic diversity and rigorous statistical analysis and modelling. We characterized 395 biofilms from neutral-alkaline hot springs spanning a 2,100km latitudinal gradient in Southeast Asia. The data clearly resolved six biogeographic regions with each defined by a core microbiome comprising specific cyanobacteria and other diverse photosynthetic, chemoheterotrophic, and chemoautotrophic taxa. Our findings demonstrated that the most influential abiotic variables (pH, conductivity, carbonate) accounted for relatively little of the observed variation in biofilm communities, and that extensive biotic interactions spanned multiple trophic levels. Importantly, we present quantitative evidence that stochasticity due to ecological drift was the most important evolutionary driver of spatial turnover at a regional scale. These insights establish n important milestone in understanding of this model system, fostering enhanced testing and comparison with more intricate microbial ecosystems.

Engineering *Escherichia coli* for saturated ether-lipid membrane formation

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Archaeal membrane phospholipids have a unique chemical composition that set them apart from bacterial glycerol phospholipids. Specifically, in archaea, the hydrocarbon chains are fully saturated isoprenoid chains ether-linked to glycerol-1-phosphate, whereas in bacteria, fatty acid chains are esterified to glycerol-3-phosphate. The pathways involved in phospholipid biosynthesis are markedly different featuring nonhomologous enzymes in the first steps. The distinction in phospholipid composition between Bacteria and Archaea is also termed the “lipid divide” and raises important questions concerning the early evolution of cellular life that emerged from the last universal common ancestor (LUCA). One hypothesis is that LUCA possessed a hybrid membrane composition, which transitioned towards distinct lipid types in both Bacteria and Archaea potentially driven by enhanced membrane stability. However, engineering of *Escherichia coli* with a mixed heterochiral membrane suggests that a such membrane might not be inherently less stable than homochiral membranes. So far, a complete biosynthetic pathway for archaeal lipids has not yet been realized in *E. coli* as the isoprenoid chains were unsaturated.

Here, we have introduced a complete pathway for isoprenoid chain saturation into the established *E. coli* model [1]. This includes an archaeal geranylgeranyl reductase (GGR) and its corresponding ferredoxin (Fd), and enzymes for Fd reduction allowing efficient saturation of phospholipid bound isoprenoid chains. Further, we examined the functional impact of archaeal ether lipid saturation on cells harbouring a mixed heterochiral membrane.

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Utilizing Extremophiles for Treating Mining-impacted Waters

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Japan has a rich history of metal mining spanning thousands of years, resulting in a large number of abandoned mines. Even after the cessation of active mining operations, these abandoned sites can persist as sources of acid mine drainage (AMD) for extended periods. Alongside the environmental challenges posed by historical mining activities, Japan also faces ongoing challenges from metal-contaminated process water generated during metal refining processes.
Our research group is investigating alternative, cost-effective technologies for the treatment of mining-impacted waters, which are often acidic and contain various heavy metals. We are exploring various remediation approaches involving direct or indirect reactions with extreme acidophiles and other microorganisms, as well as the repurposing of waste materials derived from mining-related activities.

An overview of our research progress in the following topics will be presented:

1) Biogenic scorodite formation: Removal of highly toxic As(III) through oxidative processes, facilitated by Fe-oxidising thermo-acidophilic archaea or moderately thermophilic acidophilic/ acid-tolerant bacteria. [1, 2, 3] The presentation covers the biomineralisation process and the microbial involved in this process.
2) Passive treatment of AMD: Oxidative removal of Fe(II) from AMD within a passive treatment system populated by versatile Fe(II)-oxidising acidophilic bacteria. [4]
3) Waste recycling: Utilisation of waste Fe-sludge materials consisting of Fe(hydro)oxides generated by Fe-oxidising acidophiles, as a means to remove As(III) from contaminated environments. [4]

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UV-resistant bacteria in antarctic aquatic environment and their biotechnological applications

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Antarctic aquatic microorganisms, exposed to extreme conditions of temperature, UV radiation and ice, have developed unique strategies to cope with extreme conditions and, therefore, are capable of producing potentially valuable compounds for biotechnological applications. In this context, photoprotective defense mechanisms are fundamental to counteract UV-damage due to the solar UV-B radiation including both a non-enzymatic and enzymatic antioxidant systems. In this study, 31 UV-resistant bacteria collected from different Antarctic aquatic environments (surface sea waters/ice and shallow lake sediments) were isolated by UV-C assay[1] and subsequently identified[2]. Phylogenetic analysis, based on 16S rRNA gene sequence, assigned the isolates to the Proteobacteria phylum encompassing 5 genera (*Brevundimonas*, *Psychrobacter*, *Qipengyuania*, *Sphingorhabdus*, *Sphingobium*), to Actinobacteria including 7 genera (*Kocuria*, *Gordonia*, *Rhodococcus*, *Micrococcus*, *Arthrobacter*, *Agrococcus*, *Salinibacterium*) and Firmicutes represented by 3 genera, i.e. *Staphylococcus, Mesobacillus* and *Bacillus*. Many of these bacteria showed pigmentation, suggesting that pigments, generally carotenoid-type compounds, may represent an important antioxidant defence against exposure to UV radiation in the extreme Antarctic environment. Bacterial pigments are promising and sustainable bioactive compounds, which may be used in cosmetics, food, textiles, printing, and pharmaceutical products. Antarctica, a still poorly explored resource for pigment discovery, production and applications, is a huge reservoir of pigmented bacterial biodiversity and offers promising candidates for novel chemical structures and for cell factories of bio-pigments.

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Microbial Ecology of Acidic Saline Lakes in the Yilgarn Craton Western Australia

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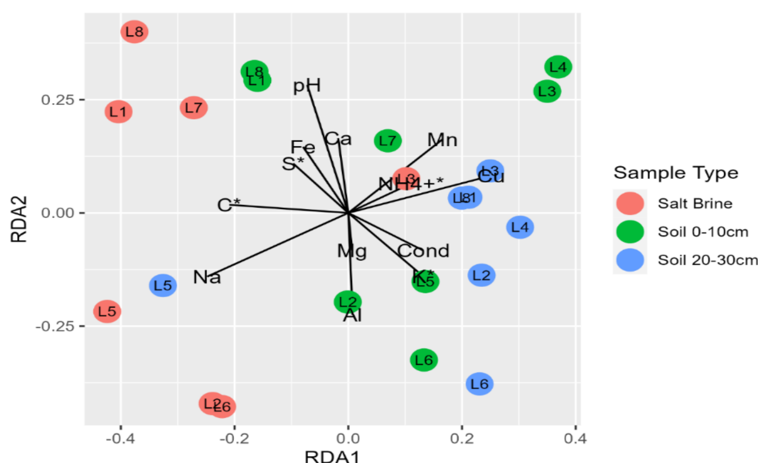
Acidic salt lakes are environments that harbor an array of biologically challenging conditions. Through 16S rRNA, 18S rRNA and ITS amplicon sequencing of eight such lakes across the Yilgarn Craton of Western Australia, we aim to understand the microbial ecology of these lakes with a focus on iron and sulfur oxidizing and reducing bacteria that have a theoretical application in biomining industries. The bacterial microbial community of these lakes revealed a large range of diversity. Redundancy analysis of soil samples revealed Na and pH were the only environmental conditions that had a significant correlation with the bacterial soil communities.



RDA analysis of the Bacterial soil and salt samples. Proportion explained, RDA1 10.6%, RDA2 9%. Adjusted R squared = 0.12. ANOVA testing of the model had a p value of 0.009. Environmental data that was significant in explaining variance by ANOVA is indicated by an asterisk.

The most abundant microbes with a hypothetical application in biomining include the genus 9M32 of the *Acidithiobacillus* family, *Alicyclobacillus* and *Acidiphilium* all of which are possible iron and/or sulfur oxidizers. It is evident through this study that these lakes harbor multiple organisms with potential in biomining industries that should be exploited and studied further.

Characterization and demonstration of thermostable carbohydrate-active enzymes for seaweed bioprocessing

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Seaweeds are an abundant and poorly utilized biomass that is rich in diverse, and often complex, carbohydrates. Seaweed polysaccharides can in theory be used as a feedstock for microbial fermentation to produce biofuels, bioplastics or other diverse products; for manufacturing of prebiotic oligosaccharides; and as a source of rare monosaccharides. Realization of these applications is however dependent on, or could benefit from, the availability of robust carbohydrate active enzymes that break down the seaweed polysaccharides.

We sought to identify and characterize thermostable enzymes that break down the seaweed polysaccharides fucoidan, ulvan, carrageenan and xylan. We screened microbial genomes and metagenomes collected from marine thermal environments for genes encoding enzymes with the respective activities. Following cloning and expression of the genes in *E. coli,* primary characterization of the enzymes was performed. In total, we characterized 12 xylanases (CAZy families[1] GH10 and GH11), 5 fucoidanases (GH107), 9 ulvan lyases (PL24, PL25, PL40) and 7 carrageenanases (GH150, GH82, GH16_17). Dozens of additional enzymes (of aforementioned GH/PL families as well as GH168 and PL37) were cloned and solubly expressed but no activity detected, perhaps due to specificity to a particular structural variant of the carbohydrates that was not tested. A large fraction of the enzymes was optimally active at high temperatures, in the most extreme cases at 85-95°C. They generally functioned optimally at circumneutral pH and in the presence of salt, in some instances at high salt concentrations.

Using the enzymes, we have developed methods for oligosaccharide production from seaweeds, and are currently developing methods for enzyme assisted extraction of proteins from the biomass.

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Life in Extreme Environments: Behavior of the Hyperthermophilic Archaeon Saccharolobus solfataricus Under Stress and Mars Simulated Conditions

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The study of life in Earth's extreme environments is a key pillar of astrobiology research. Extremophilic Archaea, which inhabiting thermal and saline springs and polar regions, provide valuable insights into how life can adapt to harsh environments, thereby redefining the concept of habitability.
In this context, the flexibility of genetic code and the genetic plasticity of extremophiles represent a key area of interest of the Astrobiology research [2]. It has been described that the expression of some genes might be regulated by a group of mechanisms globally known as "translational recoding", during which ribosomes deviate from the standard rules of translation [3]. These mechanisms reflect a phase in the genetic code evolutionary history, and it has been hypothesized that not only facilitated the transition from an ennuplet-based genetic code to one based on triplets, but also that this flexibility is a feature selected during evolution increasing the fitness of microorganisms [4].
Here we report on the response of *S. solfataricus* P2 strain under stress/simulated Mars conditions. The global response of the archaeon has been evaluated by -omics approaches, and the regulation of the expression of specific interrupted gene has been analysed. This represents one of the first study on the behavior of a Crenarchaeota under Martian simulated conditions.

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Seasonal variation of metazoans populations living in cryoconite holes

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Glacial ecosystems, particularly cryoconite holes, are dynamic habitats that harbour a range of primary and secondary producers. These microenvironments support complex biological dynamics, where metazoans such as rotifers and tardigrades are dominant[1,2]. Despite their ecological significance in recycling nutrients, little is known about the seasonal variation in the demographic dynamics of these glacier-dwelling organisms. This study aims to address this gap by exploring the seasonal population dynamics and genetic diversity of rotifers and tardigrades in cryoconite holes of Svalbard and Norway glaciers. Sediment samples were analysed in order to quantify and perform the identification with both optic and stereoscopic microscopes; further, molecular barcoding techniques were used for genotyping the mitochondrial COI gene. Significant seasonal and geographical variations in population densities were observed, with peak densities in summer and notable differences between Norwegian and Svalbard glaciers. The presence of diverse genetic clusters was found to suggest a complex ecological and evolutionary dynamic indicative of robust adaptation strategies that enable survival in extreme conditions. These findings highlight the importance of studying these unique ecosystems to understand biota's ecological interactions and evolutionary adaptations in extreme cold environments.

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Host cell binding and infection dynamics of the haloarchaeal virus HFTV1

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Archaeal tailed viruses (arTVs) are the most common isolates infecting halophilic archaea. These viruses are evolutionarily related to tailed double-stranded bacteriophages of the class Caudoviricetes. The infection cycles of the lytic viruses that infect haloarchaea are poorly understood. The cell receptors used by arTVs to bind to host cells are unknown.
Here, we study the viral life cycle of arTVs using Haloferax tailed virus 1 (HFTV1), which infects *Haloferax gibbonsii* LR2-5, as a model. A thorough description of the host properties and an in-depth characterization of the virulent life cycle allowed us to identify the key factors for attachment to the host cell. We determined the major structural proteins of HFTV1 and used electron cryo-microscopy to elucidate the structure of the virus particles and identify the elements that contribute to viral adsorption and surface binding. Uniquely, the capsid is decorated with spikes that likely mediate this initial contact. Furthermore, we were able to show that HFTV1 infection specifically targets proteins of the S-layer of the cell surface to recognize the host. RNA sequencing allowed us to gain a more detailed insight into HFTV1 infection kinetics and the viral replication cycle. Our results demonstrate that the dynamics of the host and viral transcriptome can be divided into three main phases (early, middle and late) of gene expression and that the composition of host surface proteins changes significantly during HFTV1 infection. This study significantly improves our understanding of the early infection stages of archaeal tail viruses, sheds light on the molecular interactions involved and provides a basis for further research into the mechanisms of archaeal virus-host interactions.

Microbial response of glacial microbial communities to anthropogenic and microbially produced carbon substrate amendments

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Despite the highly dilute nature of many glacial habitats, glaciers are at the center of active biogeochemical cycling, with influence across local and global scales [1,2]. Unique to glacial ecosystems is that geochemical processing is almost exclusively microbially driven. Microbial *in situ* production, combined with atmospheric deposition are the primary sources of glacial organic carbon (OC), with roughly 10^4 petagrams of OC being stored within ice worldwide. It is also evident that combustion products (in particular black carbon) are becoming a significant source of OC in glacial systems[4,5]. While it is well accepted that glaciers support diverse and active microbial communities with a range of functional capabilities, less understood is carbon transformation within glacial systems. This study investigated microbial OC processing across different glacial habitats (i.e., cryoconite, supraglacial stream water, sediment-rich ice, and clean ice) from the Juneau Icefield in Alaska using two representative endmember sources of OC 1.) algal amino acid (i.e. mimicking labile carbon produced *in situ*) and 2.) black carbon (mimicking recalcitrant aeolian deposition) as representative carbon endmember substrates. To identify translationally active microorganisms involved in processing each carbon amendment, biorthogonal noncanonical amino acid tagging was combined with fluorescence-activated cell sorting (BONCAT-FACS) and 16S rRNA gene sequencing. Between the four glacial habitats, clean ice had the lowest diversity (Shannon index 1.89; vs. 4.00-4.94). Species richness decreased during carbon substrate utilization, with distinct subsets of metabolically active microorganisms becoming evident across supraglacial environments. Taxonomic dissimilarity was greater across active populations compared to the total community samples from each environment, suggesting that the microbial populations involved in the processing of discrete OC sources is unique to each of the sampled glacial environments. Notably, cryoconite microbial populations were capable of processing both labile and recalcitrant OC sources. This study used state-of-the art techniques for the identification of translationally active uncultured organisms from cold temperature environments. Results demonstratethat glacial OC is likely microbially processed prior to export to downstream aquatic ecosystems.

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A strategic method for substantial chemical reduction via advanced forecasting modelling and high-pressure bioreactors in h_2s production management

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Microbial oilfield reservoir souring, characterised by the contamination of hydrogen sulfide in production fluids, continues to pose significant challenges to operators worldwide[1,2,3]. Understanding the likelihood and severity of sour gas production throughout the life of any oilfield asset involves computer modelling and quantitative laboratory-based simulation studies. Where significant sulfide generation is forecast, low sulfate water injection may be considered, often in conjunction with a scale mitigation treatment strategy[4]. In some cases, significant microbial activity in the downhole formation is limited due to extreme environmental conditions, restricting the geomicrobiological generation of sulfide species.

This paper will present output data from souring forecasting and modelling analysis of a high-pressure, high-temperature West African offshore asset, using the DynamicTVS© model. In addition, data from high-pressure bioreactor studies, designed to evaluate microbiological sulfide generation under high-pressure and high-temperature conditions (1,000psig to 7,000psig, and 25°C to 95°C), will be shared to support the conclusions that microbial souring would be negligible under simulated reservoir conditions.

10 years on, the asset modelled remains 'sweet' following years of observed injection water breakthrough, and the Operator continues to focus on relatively low-cost monitoring and chemical control of problematic biofouling at the topsides facilities.

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Contrasting effects of nitrogen- and phosphorus-limiting conditions on polyhydroxybutyrate synthesis in the extremely halotolerant bacterium *Halomonas elongata*

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Biodegradable polyesters like polyhydroxybutyrate (PHB) serve as an energy reserve in various bacteria, holding significant potential to replace petroleum-based plastics. PHB is recognized for its biocompatibility, thermostability, and compostability. The primary obstacle in large-scale use of PHB as a precursor for bioplastics lies in its high production costs. In a previous study[1], it was reported that *Halomonas elongata* DSM 2581^T can produce PHB at high salinity (8% w/w NaCl) on an undefined medium with glucose and yeast extract as carbon and nitrogen source. The aim of this study was to assess the PHB production by *H. elongata* using inorganic nitrogen source on a modified medium for moderately halophilic bacteria. The PHB production was evaluated using spectrophotometric-based crotonic acid assay (CAA) and fluorescence microscopy (FM) with Nile Red staining. Additionally, gravimetric assessment was employed to measure cell dry weight (CDW). Surprisingly, growth was observed in the medium with ammonium chloride (AC) and phosphate (P) (1.53 g/L in 72 hrs), but no PHB was detected by the CAA. However, FM revealed PHB inclusions in the cells. Cell morphology shifted from the characteristic elongated shape to bacilli when growth occurred on a P-limiting medium. In this case CDW was 0.50 g/L (72 hrs), lower compared to the non-limited AC/P conditions, and no PHB inclusions detection was possible by microscopy or CAA. Our study unveils a potentially greater role of phosphate in PHB production compared to nitrogen. Further investigation is required to comprehensively understand the influence of nutrients, particularly phosphate, on PHB synthesis by *H. elongata*.

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The ability of extremophilic alkaliphilic Halophilic bacteria isolated from Maharlo lake in Shiraz to remove sulfur from natural gas

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The process of separating hydrogen sulfide from natural gas is referred to as gas desalination. The analysis of natural gases containing sulfur faces the complications of chromatic separation of the gases from natural gas hydrocarbons, disturbances resulting from hydrocarbons, and the possibility of compounds in the analytical system being adsorbed.[1] As a result, gas desalination has become one of the gas industry's most significant purification processes. Modern methods of hydrogen sulfide removal are usually physio/chemical, including direct oxidation, liquid redux and adsorption, ozone oxidation, or even burning. The high cost of these methods is among their important issues given the use of catalysts and high energy consumption. A novel and efficient method which can be used for sulfide removal from rich amine and prevent it from entering to the environment, is using biological methods.

Various types of bacteria are potentially capable of sulfur processing and oxidization which are introduced as useful alternatives in acidic gas removal. Among the advantages of microbial procedures, one could mention the low investment cost, direct conversion of acid gases, and minimal waste production.[2] Maharloo lake is located 20 km from Shiraz city and has high salt. One of the sources of this lake is a sulfur spring, that's why the water of this lake has high sulfur. A Gram-positive halophilic alkalophilic bacterium isolated from Maharloo, was used in the present study. The culture medium contained 1 mole of sodium ions and pH 10, to which hydrogen sulfide was added after sterilizing the medium. Incubation was done at 37 degrees Celsius and 120 rpm for 7 to 10 days, then it was transferred from these liquid environments to solid environment. Molecular identification of isolated strains was done using PCR method This bacterium is capable of sulfur oxidization and can grow at a pH range of 8-11. The catalase and oxidase tests of the bacterium were positive. In this study, 150 halophilic, alkalophilic, sulfur-oxidizing bacteria were isolated, of which 12 strains had higher ability, and one of them had up to 99% ability to oxidize sulfur. Another application of this bacterium could be examined for indirect hydrogen sulfide removal. The Fe3O4@Zno core-shell Nano-particle was first placed in contact with a sulfur-infused medium so that adsorption took place. The exact amount of hydrogen sulfide adsorbed could be obtained through the measurement of hydrogen sulfide concentration in the solution before and after the adsorption process. Then, the adsorbent contaminated with hydrogen sulfide was added to the prepared and autoclaved culture medium, bacteria were added according to the aforementioned instructions, and the result was autoclaved in a shaker for 72 hours. Then, a super-strong magnet isolated Nano-particles from the zero-valent white sulfur produced, which was weighed using a scale after being dried. According to the research conducted in relation to the biological removal of hydrogen sulfide, all this Research has been done using Thiobacillus family bacteria and acidophilic bacteria. At The bacteria used in this research is

alkalophilic, and this is what causes this bacteria To be compatible with the conditions of industrial effluents containing hydrogen sulfide in refineries.

The results of the biological removal of hydrogen sulfide from the organic environment prove this. In the initial molecular identifications, this strain is new and has been registered in one of the prestigious microbial centers of Iran.

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Some Like It Hot: Uncovering Novel Carbohydrate-Active Enzyme from Pisciarelli Hot Spring metagenome

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Organisms that thrive in extreme environments are a rich reservoir of novel carbohydrate-active enzymes (CAZymes - www.cazy.org), characterized by a unique structure that allows them to remain stable and functional in environments such as hot springs and hydrothermal vents. These adaptations allow their use for several industrial and biotechnological applications, including second-generation biorefineries. However, many of these organisms cannot be grown in laboratory settings, limiting access to this class of biocatalysts. As a result, the *in silico* metagenomic approach has become a crucial tool for the discovery and analysis of novel CAZymes for biotechnological purposes [1,2].
In the GH122 family, currently showing 85 entries exclusively from Archaea, only the α-glucosidase from *Pyrococcus furiosus* (*Pf*-GH122) has been characterised [3,4], but the catalytic mechanism and the residues involved in the reaction remain unknown. Recently, a metagenomic study of the microbial community inhabiting the thermal springs of Pisciarelli (Naples, Italy) unveiled the entire repertoire of CAZymes in this site [1]. Among these sequences, an ORF encoding a potential GH122 that showed only 23% identity to *Pf*-GH122 was annotated and named Met-GH122. The gene expressed in recombinant form produced a functional hyperthermophilic enzyme that revealed an α-glucuronidase activity that is novel in family GH122 and distinct from its unique characterised counterpart. In addition, the comparison of the 3D-structures of *Pf*-GH122 and Met-GH122 predicted by using AlphaFold, allowed us to propose the possible molecular determinants of the different substrate specificities of these enzymes. Our data allow a new functional and phylogenetic annotation of family GH122.

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Physiological adaptations to multiple environmental parameters in an anaerobe piezotolerant from the black sea

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The lipid bilayer is a dynamic barrier, which plays a pivotal role in shielding cellular constituents against environmental fluctuations. The marine environment presents unique sorts of stressors, with a combination of as variations in nutrients concentrations, temperature, and high hydrostatic pressures (HHP). To ensure survival in challenging conditions, deep-sea microbes have evolved various strategies to uphold the integrity of their lipid membranes, particularly concerning adaptation to high hydrostatic pressure (HHP)[1].

To gain insight on such adaptations, we isolated a mesophile piezophile Bacteroidetes of the *Marinifilaceae* family, *Labilibaculum euxinus*, from 2000 m depth in the Black Sea. Analysis of the membrane lipid composition in *L. euxinus* grown in phosphorous-rich medium revealed the presence phosphorous-free lipids, notably ornithine lipids, flavolipins and capnine lipids, which are usually associated with adaptation to phosphate limitation.

We tested the resilience of this membrane to variations in phosphate concentration and HHP by growing *L. euxinus* at various phosphate concentrations and hydrostatic pressure. While morphological analysis revealed elongated cells, our lipid analysis uncovered a surprisingly robust membranes which present minimal changes in the tested conditions. Our results highlight the wide range of adaptations that bacteria are capable of, and reshaping our understanding of environmental stress dynamics.

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Extracellular electron transfer drives efficient H_2-independent methylotrophic methanogenesis by *Methanomassiliicoccus*, a seventh order methanogen

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Methylotrophic methanogenesis is achieved via methyl group dismutation or H_2 reduction [1]. This study reports extracellular electron droving efficient methylotrophic methanogenesis. The 7th order methanogen *Methanomassiliicoccus luminyensis* exclusively implements H_2-dependent methylotrophic methanogenesis, but strain CZDD1 isolated from paddy soil possessed a higher methane-producing rate in coculture with *Clostridium malenominatum* CZB5 or the electrogenic *Geobacter metallireducens*. Chronoamperometry detected current production from CZB5, and current consumption accompanied CH_4 production in a methanol-containing electrochemical culture of CZDD1. This demonstrated that M. luminyensis was capable of both direct species electron transfer (DIET) and extracellular electron transfer (EET) in methylotrophic methanogenesis. EET and DIET also enabled CZDD1 to produce methane from dimethyl arsenate. Differential transcriptomic analysis on H_2- versus EET- and DIET-cocultures suggested that a membrane-bound Fpo-like complex and archaella of M. luminyensis CZDD1 could accept extracellular electrons. Given the ubiquitous environmental distribution of Methanomassiliicoccus strains, EET driven methylotrophic methanogenesis may contribute significantly to methane emission.

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Involvement of capsular polysaccharide in the biogenesis of extracellular membrane vesicles Of *Shewanella vesiculosa* hm13

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Extracellular membrane vesicles (MVs) are nano-sized particles released by many bacteria, which have been attracting a lot of attention because of their physiological importance and great potential in biotechnological applications. *Shewanella vesiculosa* HM13, a psychrotrophic Gram-negative bacterium isolated from the intestinal contents of horse mackerel, abundantly produces MVs that carry a single major cargo protein P49. This protein is loaded onto MVs through interaction with capsular polysaccharides (CPS) on the surface of MVs. *S. vesiculosa* HM13 is expected to be useful as a host for producing thermolabile proteins using MVs as a platform.
To elucidate the mechanism of MV production and promote its application, we performed transposon (Tn)-mediated random mutagenesis of *S. vesiculosa* HM13 and screened the mutant library. As a result, two mutants with significant hypo-vesiculation phenotype were obtained. Surprisingly, analysis of these mutants revealed that the hypo-vesiculation phenotype was not caused by Tn-insertion but by spontaneous mutations that occurred in a common gene, *hm3339*. This gene and its nearby genes were predicted to encode glycosyl transferases. Their involvement in MV production was confirmed by gene disruption experiments, and their disruption also resulted in dissociation of P49 from MVs. We found that disruption of *hm3339* caused a reduced CPS content of cells and a reduced negative charge on the cell surface. Notably, abnormal bubble-like structures were found on the cell surface of Δ*hm3339*. These results highlighted the unexplored function of CPS in the biogenesis of MVs.

Novel ammonification mechanism in *Acididesulfobacillus acetoxydans*, coupled to NO detoxification at low pH

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The biological route of nitrate reduction has important implications for the bioavailability of nitrogen within ecosystems. Nitrate reduction via nitrite, either to ammonium (ammonification) or to nitrous oxide or dinitrogen (denitrification), determines whether nitrogen is retained within the system or lost as a gas. The acidophilic sulfate-reducing bacterium (aSRB) *Acididesulfobacillus acetoxydans* can perform dissimilatory nitrate reduction to ammonium (DNRA). While encoding a Nar-type nitrate reductase, *A. acetoxydans* lacks recognized nitrite reductase genes. In this study, *A. acetoxydans* was cultivated under conditions conducive to DNRA. During cultivations, we monitored the production of potential nitrogen intermediates (nitrate, nitrite, nitric oxide, hydroxylamine, and ammonium). Resting cell experiments were performed with nitrate, nitrite, and hydroxylamine to confirm their reduction to ammonium, and formed intermediates were tracked. To identify the enzymes involved in DNRA, comparative transcriptomics and proteomics were performed with *A. acetoxydans* growing under nitrate- and sulfate-reducing conditions. Nitrite is likely reduced to ammonia by the previously undescribed nitrite reductase activity of the NADH-linked sulfite reductase AsrABC, or by a putatively ferredoxin-dependent homolog of the nitrite reductase NirA (DEACI_1836), or both. Remarkably, we identified a nitrosamine stress detoxification mechanism, particular from low pH. In nitrate conversion, extracellular nitrite disproportionates into nitric oxide, which can freely permeate the membrane and enter the cell. Cytoplasmic nitrite can be converted (as side activity) by NarG into nitric oxide. Cytoplasmic nitric oxide can be reduced to nitrous oxide by Hcp, which is subsequently reduced to dinitrogen gas by NosZ (and NosFYD) with PetABC. In this way, toxic generated NO is reduced to dinitrogen gas. In conclusion, this study increases our knowledge about ammonification routes, helping the interpretation of (meta)genome data from various ecosystems on their DNRA potential and the nitrogen cycle. Besides, reveal nitrosamine stress detox mechanisms at low pH.

Nuances of type III CRISPR-Cas systems in *Thermus thermophilus*

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Bacteriophages of extremophilic bacteria are diverse in shapes of their virions, in infection strategies, and in sizes of their genome varying from roughly 6k bp (phage Zuza8) to more than 80k bp (phage P23-45). Respectively, a variety of bacterial anti-viral systems engage in the never-ending arms-race between bacteria and phages.

CRISPR-Cas systems have been found throughout the whole domain of prokaryotes and also found in extremophiles, such as thermophilic bacteria *Thermus thermophilus*. The type III CRISPR-Cas stands out among other defence systems with its unique set of biochemical activities[1]. The effector complex possesses targeted ssRNase activity and ssDNase (via HD domain), while auxiliary nucleases were found to have non-specific RNase and nickase activities[1]. Auxiliary nucleases are activated in trans by cyclic oligoadenylate compounds that are produced by the cyclase PALM domain of Cas10 upon detection of viral RNA[1]. It is yet to be understood which of this diverse set of activities is crucial for the overall response of type III CRISPR-Cas system. Notable, HD domain is present only in type III-A CRISPR-Cas system, resulting in controversy surrounding its function[1].

Recent developments allowed us to repurpose the already present type I CRISPR-Cas system of *Thermus thermophilus* to genetically edit its own genome. Using such a powerful tool, we genetically dissect type III-A and type III-B CRISPR-Cas systems of *Thermus thermophilus* HB27 strain to understand which activities and which genes are needed for plasmid interference and which – to fight against different phages.

Enzymatically inactive PALM domain leads to a complete loss of interference against any plasmid or phage. Unexpectedly, mutation in the HD domain in III-A system, which is notably absent in III-A system, renders it inactive against phages but not against plasmids.

This research also investigates the role of each different auxiliary gene and its interplay against plasmids and phages.

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Extremophilic cell platforms for CO_2 valorization into pharm and cosmetic ingredients

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The transformation of CO_2 off-gas emissions into valuable compounds represents a key target for the development of a sustainable and circular bioeconomy. In this study, we explored for the first time a novel market opportunity based on the conversion of CO_2 into ectoine (1000 €/kg) and hydroxyectoine (1200 €/kg), osmolytes produced by prokaryotes in high salinity environments[1]. To this aim, halophilic microbes able to use CO_2 as a carbon source and H_2 as a green-energy source were identified by databases and their genomes mined for the genes of ectoines synthesis pathways (*ectABCD*)[2]. A total of 11 species had the genes to synthesize ectoines fixing CO_2 aerobically. Further laboratory analyses in 1 L batch bioreactors fed with 10% of CO_2 showed that the most promising bacteria for the implementation of this novel bioconversion process were *Hydrogenovibrio marinus*, *Hydrogenibacillus schlegelii* and *Rhodococcus opacus*. After salinity and H_2/CO_2/air ratio optimization, *H. marinus* accumulated the highest amount of ectoine among the three tested strains at 6% NaCl (79.6 ± 10.5 mg ectoine g biomass^{-1}) and had the fastest growth with the shortest lag phase. Notably, ectoine content was similar for *R. opacus* and *H. schlegelii* at all the salinities tested, but hydroxyectoine levels increased at higher salinities. *R. opacus* accumulated maximum hydroxyectoine values at 7% NaCl (52.1 ± 4.3 mg hydroxyectoine g biomass^{-1}) whereas *H. schlegelii* achieved maximum content at 5% (62.0 ± 7.9 mg of hydroxyectoine g biomass^{-1}). These hydroxyectoine values fall within the range of yields obtained from rich carbon sources and were obtained as isolated osmolytes. Overall, this research set, by means of the use of an innovative genomic technique and bioengineering, the possibility to find novel microorganisms able to transform CO_2 into fine chemicals with important value for the economy and society.

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A thermophilic TnpB nuclease enables flexible single-nucleotide editing with templated repair in archaea and bacteria

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TnpBs encoded by the IS200/IS605 family transposon are among the most abundant prokaryotic proteins from which type V CRISPR-Cas nucleases may have evolved [1]. Since TnpBs can be programmed for RNA-guided dsDNA cleavage in the presence of a transposon-adjacent motif (TAM) [2, 3], these nucleases hold immense promise for genome editing. However, the activity and targeting specificity of TnpB in homology-directed gene editing remain unclear. Here we report that a thermophilic archaeal TnpB enables efficient gene editing in the natural host. Interestingly, the TnpB has different TAM requirements for eliciting cell death and for facilitating gene editing. By systematically characterizing TAM variants, we reveal that the TnpB recognizes a broad range of TAM sequences for gene editing including those that do not elicit apparent cell death [4]. Importantly, TnpB shows a very high targeting specificity on targets flanked by a weak TAM. Taking advantage of this feature, we successfully leverage TnpB for efficient single-nucleotide editing with templated repair in the natural host and in mesophilic bacterial hosts. The use of different weak TAM sequences not only facilitates more flexible gene editing with increased cell survival, but also greatly expands targeting scopes, and this strategy is probably applicable to diverse CRISPR-Cas systems.

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Biotechnological potentialities of exopolymers produced by *bacilli* of shallow hydrothermal vent origin

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Polyextremophilic bacteria from shallow hydrothermal vents of the Eolian Islands (Italy) are ideal candidates to experimentally address questions either to extend our knowledge on the resistance strategies under extreme environmental conditions, such as low pH values, high temperatures, high concentrations of CO_2, H_2S, heavy metals and hydrocarbons, and to prospect their use for biotechnological purposes [1,2]. Thermophilic and thermotolerant bacilli from these sites are able to produce biomolecules (e.g., exopolysaccharides and lipopeptides) that could offer them several advantages to cope environmental stresses, including the ability to adhere and develop structured biofilm on different surfaces, maintain the proper hydration and nutrient availability, decrease heavy metal toxicity, and contrast predators and pathogens. These biopolymers possess unique chemical and physical properties, mainly thermostability, and related biological activities (non-cytotoxicity, anti-cytotoxicity, and biodegradability), that make them attractive in different applicative sectors. Among them, exopolysaccharides (EPS) and biosurfactants (BS) produced by *Bacillus licheniformis* B3-15 *and B. horneckiae* APA and SBP3 have been reported among the few compounds derived from marine bacteria with antiviral and immunomodulatory activities. Without exert any antibacterial effects, EPSs and BSs at low concentration prevent biofilm formation of human pathogens (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*), acting as anti-adhesives on different substrata. Due to their chemical composition, the functional groups of EPS and BS are involved in the absorption of heavy metals (arsenite, arsenate, mercury and vanadium) and their toxicity reduction. Due to their hydrating properties, EPSs and BS could also useful to contrast desiccation and improve soil quality. With ongoing research on their properties and their related biological activities, biopolymers from Eolian bacilli could be useful in responding to the increasing demand for novel bioproducts in medical and non-medical applications.

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Xyloglucan oligosaccharides hydrolysis exploration by three exo-glycosidases from *S. solfataricus*

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Hyperthermophilic microorganisms have always been an invaluable source of thermostable enzymes useful for the development and improvement of bio-based technologies. In the field of biocatalysis and the transition towards a bio-based economy, enzymes acting on lignocellulose biomasses have garnered significant attention. However, the study of the catalytic activity of lignocellulose-degrading enzymes needs to be improved to achieve efficient hydrolysis of plant biomass[1]. In this context, hemicellulases from hyperthermophilic archaea show promising features as biocatalysts and provide many advantages in industrial applications due to their stability under the harsh conditions encountered during the pretreatment process. Nevertheless, archaeal hemicellulases are less studied compared to their bacterial counterparts, and the activity of most of them has barely been tested on natural substrates[2]. Here, we investigated the hydrolysis of xyloglucan oligosaccharides from two different plant sources using, both synergistically and individually, three glycoside hydrolases from *Saccharolobus solfataricus*: a GH1 β-gluco-/β-galactosidase, an α-fucosidase belonging to GH29, and an α-xylosidase from GH31. The results showed that the three enzymes were able to release monosaccharides from xyloglucan oligosaccharides after incubation at 65°C. The concerted action of the β-gluco-/β-galactosidase and the α-xylosidase on both xyloglucan oligosaccharides were observed, while the α-fucosidase was capable of releasing all α-linked fucose units from xyloglucan derived from apple pomace. This represents the first GH29 enzyme from subfamily A that is active on xyloglucan.

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A Comparative Study of Cesium Ion Resistance in alkaliphilic *Microbacterium* sp. TS-1 and *Escherichia coli* ZX-1

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Our study comprehensively overviews microbial strategies against Cs^+ toxicity, contrasting the naturally evolved Cs^+ resistance in alkaliphilic *Microbacterium* sp. TS-1 [1] with the spontaneously acquired resistance in *Escherichia coli* strain ZX-1 derived from *E. coli* Mach1 $T1^R$ (F^- delta *lac*X74 *hsdR*(r_k^- m_k^+) delta *rec*1398 *end*A1 *tonA*; Invitrogen) [2]. *Microbacterium* sp. TS-1, isolated from Jumping Spider ground extract, exhibits intrinsic resistance to very high Cs^+ concentrations (up to 1200 mM CsCl), primarily through a novel Cs^+/H^+ antiporter named CshA and a magnesium transporter, MgtE [3,4,5]. These mechanisms effectively lower intracellular Cs^+ concentrations, highlighting their potential for bioremediation applications. *E. coli* ZX-1, resistant to up to 700 mM CsCl, is a model for studying induced Cs^+ resistance mechanisms through mutational analysis and genetic engineering. By exploring the genetic adaptations in ZX-1 and the natural resistance in TS-1, we highlight the synergy between biological engineering and natural microbial resilience. This synergy offers novel insights for developing effective bioremediation agents to clean cesium-polluted environments, showing promise for advancing microbial strain development for environmental restoration.

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A novel contractile injection system in extremophilic bacteria

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Bacterial contractile injection system (CIS) is a syringe-like protein complex whose basic organization and action resemble a bacteriophage tail. The CIS's mode of action is tightly linked to its intracellular localization. For example, type six secretion system is a bacterial CIS anchored to the cell envelope. This system translocates a toxic effector from the cell to an adjacent neighbouring cell, and it is often employed for interspecies bacteria competition. Recently discovered cytoplasmic CIS in *Streptomyces* species is a self-targeted system that exists in the cell cytoplasm as "floating" particles and releases an effector under cell stress to induce cell death.
The CIS genes are found in bacteria living in a broad range of econiches including hot spring mats. However, it is challenging to predict CIS's intracellular localization using only sequencing data. To overcome this challenge, we have developed a cryo-electron tomography workflow as a technique complementing metagenomics and proteomics. Additionally, we developed an immuno-electron microscopy protocol to identify and quantify CIS particles in environmental samples. Using this approach, we discovered a novel bacterial CIS in thermophilic multicellular *Chloroflexota* bacteria populating hot spring mats worldwide. We found that this system is similar phylogenetically and structurally to the cytoplasmic CIS in multicellular *Streptomyces.* Interestingly, using our approaches, we have discovered that *Chloroflexota* cells produce different numbers of CIS particles depending on the mat micro-niches they occupy. In agreement with this, we observed that CIS was also non-constitutively expressed under laboratory conditions. Motivated by this discovery, we searched and analysed similar CIS in extremophilic bacteria from other bacterial lineages. Overall, we have gained an understanding that bacterial cytoplasmic CIS is an overlooked cellular feature of the extremophilic bacteria. Potentially CIS can be involved in the cell fate control or intraspecies interaction among extremophilic bacteria within a microbial community.

Thermococcus barophilus adapts to high hydrostatic pressure by modulating its metabolism

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Life would have arisen, in line with a plausible hypothesis, in the deep sea, sheltered from the harmful radiation of the young star (Sun), and close to sources of energy (e.g. hydrothermal vents) likely to fuel oxidation-reduction reactions. It would therefore have appeared in conditions of high hydrostatic pressure (HHP). Modern organisms are adapted to HHP (piezophiles) and have a physiology that depends on pressure; however, understanding their adaptation to HHP remains a major challenge because it is often co-evolved with other environmental adaptations. However, the first demonstration of structural adaptation of the proteome in piezophiles comes from whole-cell comparative studies between two nearly isogenic piezophilic and piezosensitive micro-organisms, namely *Thermococcus barophilus* and *Thermococcus kodakarensis*, belonging to the order Thermococcales, which share identical growth characteristics, with the exception of adaptation to HHP[1]. Investigation of *T. barophilus* as a model organism for piezophilic hyperthermophiles has also demonstrated that HHP adaptation in this organism consists of the repression of several amino acid (AA) biosynthesis genes, leading to cells that are auxotrophic for up to 17 AA at HHP[2], whereas acquiring AA at HHP in *T. barophilus* is likely to rely on the secretion of proteases enabling the degradation of polypeptides from which the breakdown products might be used by the cell. The modulation of metabolism in *T. barophilus* by HHP, concerns also energy and carbohydrate metabolisms[3,4], both of which are positively regulated by HHP via transcriptional regulators. Recent results obtained by combining genetic, physiological and omic approaches will be presented in order to further our knowledge of the mechanisms of adaptation to HHP in *T. barophilus*.

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Trace metal availability controls metabolic shifts in microorganisms

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Trace elements are fundamental for the growth of all life forms on this planet. They are used as cofactors in proteins and enzymes, playing key roles in energy conservation and redox chemistry[1]. Life uses selected trace elements to drive and control specific reactions. In particular, energy conserving reactions (redox reactions) require diverse elements as key catalytic centers to be tuned to the midpoint potential of their substrate[2,3]. Metals such as Fe, Co, Ni, Zn, Mo, W, V, and Cu are used in these proteins and constitute essential micronutrients for microbial growth[3]. While the control imposed on microbial metabolism by the scarcity of some of these metals in the environment is well known, for example for iron scarcity in the ocean controlling primary productivity, the relationship between trace element availability and microbial metabolic diversity has not been explored in detail[4]. Given that the availability of many of the essential trace elements has changed as a function of changing planetary conditions in deep time, unveiling the link between trace element availability and functional diversity might shed light on the evolution of metabolism and the emergence of biogeochemistry[3,4]. Here, we will present recent data from field and laboratory experiments elucidating the impact of trace element availability on microbial functional diversity, showing that trace elements can be used to manipulate microbial metabolisms controlling the shift between diverse electron acceptors. We further show that this mechanism is widespread in the tree of life and that the scarcity of key trace elements imposes additional metabolic costs on the fitness of microorganisms.

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Differential gene expression of $scCO_2$ tolerant *Priestia megaterium* SR7 under variable growth rate and end-product toxicity during continuous flow under an ambient atmosphere

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Microbes are a main source of production for industrial bioprocesses such as biofuel and biosurfactant production[1]. With an increasing range of bioprocesses occurring in harsher environments, there is a demand for new extremophile model organisms that demonstrate tolerance toward adverse conditions and end-product toxicity. *Priestia megaterium* SR7 was isolated from the McElmo Dome CO_2 field and was shown able to grow in an aqueous phase under supercritical CO_2 headspace and able to be engineered to produce isopentanol and isobutanol[2]. To better understand the growth and tolerance of *Priestia megaterium* SR7 to the toxicity of potential target end products, the species is grown in replicates in chemostat culture and sampled under steady state conditions with varying growth rates and the addition of inhibitory end products. Samples are analysed for differential gene expression, physiological differences, and metabolome changes. Results show significant variation in profiles between higher and lower growth rates with differences in stress response, sporulation, flagella motility and amino acid production pathway intermediates. A varying tolerance towards the addition of inhibitory branched alcohols is also observed. The development of a model organism for production entails maximizing growth and production, thus the need for detailed understanding of the growth-rate dependent physiology, gene expression and tolerance towards end-product toxicity.

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A new archaeal Amylomaltase from metagenomic dataset of extreme environments

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Amylomaltases belong to Glycoside hydrolase (GH) family GH77 [1], which is monospecific with the 4-α-glucanotransferase activity (EC 2.4.1.25) detected both in prokaryotes (amylomaltases) and in plants and algae (named disproportionating enzymes, or D-enzymes). They hydrolyse glucosidic bonds of α-1,4′-D-glucans and transfer the glucan portion with the newly available anomeric carbon to the 4′-position of an α-1,4′-D-glucan acceptor. The interest in amylomaltases is due to their potential biotechnological applications, such as the production of sugar substitutes, cycloamyloses, which could serve as carriers for hydrophobic molecules and thermoreversible starch gels, offering alternatives to animal gelatin substitutes [2]. Despite their significance, few GHs from thermophilic microorganisms have been characterized compared to the large number (>18,000) of available GH77 sequences. This is due to the increasing availability of (meta)genomic datasets, also obtained from extreme environments. Therefore, is of utmost importance to biochemically characterize the GHs encoded by metagenomic sequences already available. Here, we report on the biochemical characterization and analysis of the disproportionation activity reaction products of a novel member of GH77 identified in the metagenomic dataset from a hot mud/water pool (T = 85 °C; pH 5.5) in the solfataric field of Pisciarelli, Agnano (Naples, Italy) [3].

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Exploiting CRISPR in *Thermoanaerobacter kivui*

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Acetogens are autotrophic microbes, attractive for industrial, carbon-negative production of value-added chemicals. *Thermoanaerobacter kivui* is a fast-growing, thermophilic acetogen, able to utilize gaseous C1 compounds, such as CO_2, CO (after adaptation), and H_2, which are available in large quantities as industrial steel mill off-gasses or can be obtained via gasification from biogenic residues. Combined with its genetic tractability, *T. kivui* is an ideal host for metabolic engineering towards industrial production of chemicals of interest.

Current genome editing methods rely on homologous recombination with auxotrophy-based selection, which requires multiple subculturing steps and can be labor-intensive. To fully exploit *T. kivui* potential for industrial biotechnology, developing more efficient genome editing tools would be desirable.

In this work, we created a genome editing method based on the endogenous CRISPR Type I-B system of *T. kivui*. With this method, a deletion (*pyrE* used as the proof-of-concept) or insertion (pFAST) is possible in a single plating step with a transformation efficiency of 10^6 CFU/µg/ml and 100% editing efficiency. With this tool, the speed of genome editing in *T. kivui* was drastically accelerated, with a current average speed of 1 genome-edited strain per month, which is also increasing. These results render *T. kivui* endogenous CRISPR-based genome editing the most efficient among the thermophilic anaerobes. Additionally, conventional hosts used for plasmid cloning, such as *E. coli*, can be bypassed without loss in efficiency, as the assembled DNA fragments can be transformed directly to *T. kivui*.

The redox-sensing factor *rex* and the heat-responsive transcriptional repressor *hrcA* were targeted with CRISPR for deletion, and phenotypic analysis of the resulting mutants was performed to illuminate the role of these transcription factors in *T. kivui*. Overall, the developed highly efficient CRISPR system should facilitate iterative genetic engineering approaches in the future and could potentially be applied to other thermophilic hosts.

Investigating the surface glycans from the psychrotolerant bacterium *Shewanella vesiculosa* hm13

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Extracellular Membrane Vesicles (EMVs) are small spheres (20–250 nm) that, when released by the bacterial membrane of Gram-negative bacteria, comprise phospholipids, lipopolysaccharides (LPSs), proteins, peptidoglycans, DNA, and RNA.[1] EMVs play different roles in the physiology and pathogenicity of bacteria: biofilm formation, toxin delivery, antibiotic resistance, immunomodulation, stress response, horizontal gene transfer and communication among cells and species. Many studies addressing the biogenesis and the potential application of EMVs have been performed.[2] In contrast, the physicochemical properties of these vesicles have been poorly investigated.
Here, we reported the identification and the detailed structural characterization of the LPS and capsular polysaccharides from the psychrotolerant bacterium *Shewanella vesiculosa* HM13 by NMR spectroscopy and chemical analyses. This bacterium produces a large amount of extracellular membrane vesicles, carrying a major protein P49, the loading of which seems to be influenced by the glycans decorating the membrane.
Here we report the structural characterization, using chemical analyses and NMR spectroscopy, of the surface glycans isolated from the *wzx*- and *nfnB*-mutant strains of *S. vesiculosa* HM13, which are unable to load P49 on the membrane vesicles. [3,4]

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Evolving thermophiles to thrive at suboptimal temperatures

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Phylogenetic studies suggest that LUCA, the last universal common ancestor of Bacteria and Archaea, was a thermophile.[1] This implies that at some point in the history of Early Earth, mesophilic organisms may have evolved from thermophilic organisms, but laboratory experiments towards that are missing.

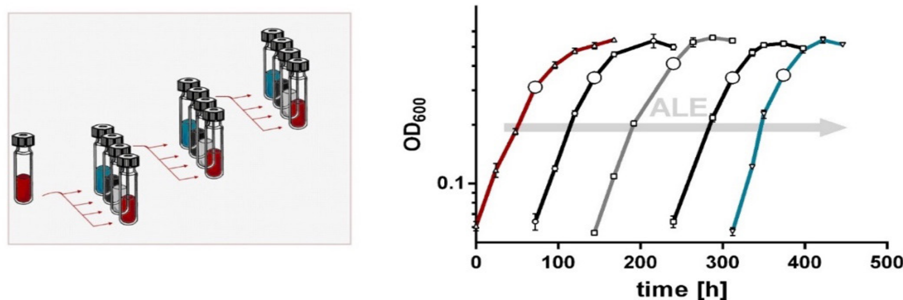

Fig.: 1 Exemplary ALE workflow (data from *T. maritima*, 50 °C)

We used the hyperthermophilic bacterium *Thermotoga maritima* and the acetogenic bacterium *Thermoanaerobacter kivui* our adaptive laboratory evolution approach (Fig. 1). *T. maritima* was chosen as representative of the *Thermotogales,* which contains mesophilic and thermophilic members with broad temperature ranges[2]. It has been hypothesized that the former may have evolved from the latter within the order.[3] *T. kivui* was chosen for its putative ancient physiology.
Characterization of both organisms revealed their ability to grow below the published minimal temperature increasing the temperature range of these organisms by at least 10 K. For *T. maritima* growth at lower temperatures was accompanied by dramatic changes in morphology, with larger cells compared to their counterparts grown at 80 °C. We analysed fermentation products at different temperatures and observed higher concentrations of lactate during incubation at 45 and 50 °C. Adaptive laboratory evolution led to strains with higher growth rates at certain lower temperatures. For *T. kivui* a new optimal temperature of 60 °C was observed after approximately 180 generations of serial passaging at 45 °C[5]. Currently, we are studying the molecular basis for these phenotypes. We anticipate that these adaptations will allow insights into the evolution of thermophiles towards lower temperatures.

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A novel alcohol dehydrogenase from *Hyperthermus butylicus*

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Hyperthermus butylicus is a hyperthermophilic crenarchaeon that grows between 95°C and 106°C and produces 1-butanol as an end product[1]. A thermostable *H. butylicus* alcohol dehydrogenase (HbADH) is required for being the key enzyme responsible for this production; however, there is only one annotated gene that encodes the zinc-dependent ADH (HbADH1) and it shows no catalytic properties required for the production of 1-butanol at high temperatures[2]. A novel ADH, named as HbADH2, was purified from a cell-free extract of *H. butylicus*, and its characteristics were determined[3]. The gene that encodes HbADH2 was determined to be HBUT_RS04850 and annotated as a hypothetical protein in the genome of *H. butylicus*. HbADH2 was found to be a primary-secondary ADH capable of using a wide range of substrates, including butyraldehyde and butanol. Butyraldehyde had the highest specificity constant (k_{cat}/K_m). The apparent K_m values for other substrates, including ethanol, 1-propanol, 2-propanol, butanol, acetaldehyde, propanal, and acetone, were also determined. The optimal pH values for catalyzing aldehyde reduction and alcohol oxidation were 6.0 and 9.0, respectively, while the optimal temperature was higher than 90°C. Based on its substrate specificity, enzyme kinetics, and thermostability, HbADH2 may be the ADH that catalyzes the production of 1-butanol in *H. butylicus*. The putative conserved motif sites for $NAD(P)^+$ and iron binding were identified by aligning HbADH2 with previously characterized Fe-containing ADHs.

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Cold active biosurfactants: An adaptive strategy for enhanced survival across environmental extremes

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Biosurfactants are biologically produced amphipathic compounds composed of a hydrophilic head and a hydrophobic tail. This chemical composition allows surfactants to reduce the interfacial tensions between liquids, solids, and gases. While the investigation of cold active biosurfactants from psychrophilic microorganisms is early on, the contribution of cold active biosurfactants to sustainable biotechnologies has proven effective at significantly reducing environmental impacts[1,2]. Cold active biosurfactants, compared to their synthetic counterparts, show greater biodegradability, low toxicity, high-foaming properties, and high stability across pH, temperature, and salinity ranges. However, little is known about the advantages provided by biosurfactants to the survival or proliferation of microorganisms in the environment[1]. This yields the question- what role do biosurfactants play in microbial survival and growth under environmental extremes? In this study, we investigated the role of biosurfactant production by an Antarctic bacterial
isolate (Serratia sp. PL17). To demonstrate biosurfactant effects, we generated a genetically engineered biosurfactant deficient mutant to run in parallel to all wildtype experiments. A range of temperature, pH, salinity, and the presence of hydrophobic water insoluble substances (*i.e.*, petroleum hydrocarbons) were performed for two experimental designs to: (i) closely recapitulate biosurfactant production concentrations in the environment (wildtype vs. biosurfactant deficient mutant) and (ii) the impact of higher biosurfactant concentrations (wildtype and mutant spiked with purified biosurfactant). The role of biosurfactants was determined as a measure of metabolic activity (*i.e.*, biomass production/maintenance or growth). We also characterized the temporal biotic and abiotic effects of this cold active biosurfactant on the chemical properties of petroleum hydrocarbons using gas chromatography mass spectrometry (GC–MS). Results indicate that biosurfactants, identified as a cyclic lipopeptide, lead to enhanced survival (i.e., enhanced cellular activity) in environmental extremes and increased hydrocarbon biodegradation under conditions representative of cold temperature environments. The detailed discussion will be presented at the meeting.

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Cofactor and amino acid metabolism in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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Thermococcus kodakarensis is a hyperthermophilic archaeon isolated from Kodakara Island, Japan. The organism is an obligate heterotroph and anaerobe, utilizing a wide variety of organic compounds including polysaccharides, 2-oxoacids, amino acids and peptides as carbon and energy source. We are interested in the metabolism of this archaeon, and have been examining the enzymes and pathways involved in the catabolism and biosynthesis of sugars, cofactors and amino acids.
We took interest in a number of genes whose protein products could potentially be involved in biotin biosynthesis. This was not the case, and two were demonstrated to be involved in lipoate biosynthesis, encoding a structurally novel lipoyl synthase. Another turned out to encode an ornithine ω-aminotransferase. Genetic studies on ornithine ω-aminotransferase indicated that ornithine acts as a precursor for proline biosynthesis. In the search for enzymes involved in the supply of ornithine, we identified enzymes that would supply ornithine from arginine. The pathway converts arginine, ADP and phosphate to ornithine, ATP and carbamoyl phosphate via citrulline. The initial reaction is catalyzed by an enzyme we designate arginine synthetase. Properties of the enzyme and the pathway directing arginine to ornithine will be presented.

Novel low molecular weight thiols in Nitrospiraceae

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All aerobic organisms face oxidative stress, this can lead to reactive oxygen species (ROS) and cell damage. Intracellularly, ROS are quenched by antioxidant systems, among those low molecular weight (LMW) thiols are found[1]. LMW thiols manage oxidative stress, keep a reduced cytoplasm, and chelate metals, just to name a few of their functions[2]. These small molecules differ structurally among microorganisms and not all microorganisms have a known LMW thiol[3]. In this work we evaluated unknown LMW thiol of two members of the Nitrospirae class, the bioleaching microorganism *Leptospirillum* sp. CF1 and *Nitrospira marina*, the nitrite/ammonia-oxidizing genera. The genus *Leptospirillum* is one of the most renowned bioleaching genera being able to dominate natural and industrial environments[4]. Same is true for *Nitrospira,* it is fundamental for nitrification to remove nitrogen in water systems[5]. It has been previously reported that Nitrospirae class lacks any of known LMW thiol biosynthetic genes[3], furthermore *Leptospirullum* extracts lacks glutathione reductase activity[6]. In this study, using the fluorescent probe monobromobiname (mBrB), we have determined the presence of novel LMW thiols in these species. They both possess 3 new LMW thiols, but they only share 2 in common. The response to different oxidative stressing agents (Fe^{3+} and diamide) was evaluated on *Leptospirullum* and was observed that these 3 thiols responded differentially to the stresses. This work lays fundamental work for the characterization and the study of these new thiols in these extremophilic organisms.

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Sustainable degradation of keratin waste using thermophilic enzymes: thermok

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The poultry industry faces a growing challenge: the sustainable management of its escalating feather waste. Traditional methods like landfilling and incineration are environmentally unfriendly. Microbial keratinases offer a promising solution for degrading keratin which is the main protein in feathers[1].
ThermoK is a consortium project funded by the European Research Area Network Cofund (ERA-NET Cofund) Food Systems and Climate (FOSC) involving partners in Norway, France, South Africa and the UK. At the University of Exeter Biocatalysis Centre, we contribute by focusing on the biochemical and structural analysis of different proteolytic enzymes shown to be active in feather degradation. Utilizing our in-house database of thermostable enzymes (identified through genomic and metagenomic analyses) in conjunction with proteomics data, derived from the feather degradation experiments of our partners, we are identifying and cloning the most promising candidates.
Promising candidates, including a thermophilic pyrrolidine carboxypeptidase[2], two endopeptidases and a disulfide bond reductase, are being expressed, purified, and rigorously tested for keratin degradation. These studies aim to not only develop a sustainable solution for feather waste but also unlock valuable byproducts including peptides and amino acids for use in fertilizers, animal feed, cosmetics, and pharmaceuticals.

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Discovery of Novel Glycosyl Hydrolases from Extreme Environments: Insights from Magma Degassing at Vulcano Island

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The extreme environments such as deep-sea hydrothermal vents, acidic hot springs, and alkaline hypersaline lakes, are populated by microorganisms adapted to thrive under harsh conditions, possessing enzymes with exceptional biochemical properties[1]. This work focuses on the application of metagenomic techniques to uncover novel GHs from two samples of soil from the extreme environment of Vulcano island (Italy). Samples were taken at the acidic SO_2-bearing fumaroles with temperatures of ≥100°C at the La Fossa cone, during the 2021 unrest, on November. We investigated the microbial communities and their functional potential by using KBase[2]. Mineralogical and chemical composition have been determined by convectional petrological techniques with the aim to unravel eventual relations between inorganic and organic signatures. We here show that the communities in the two sulfurous soil samples at temperature of ca. 100°C (Fum5 and Fum7) diverge significantly and show different phyla and abundance profiles. We obtained 23 and 19 metagenome-assemble genomes (MAG)s from Fum5 and Fum7, respectively. MAGs selected for further evaluation included members of the genus *Acidithiobacilla* with the purpose of revealing their functional potential in the production of nanocellulose. The results integrate those in the literature and could have a volcanological relevance in the future.

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Post-capture desorption of CO_2 via Extremophilic amidases

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Chemical absorption of CO_2 from industrial flue gas streams via alkanolamines, such as monoethanolamine, in aqueous solutions is a highly mature and well-established process, however the high heat input requirement for the post-capture stripping of CO_2 is a major drawback. Temperatures around 100-140 °C should be reached to break the C-N bond in the resulting carbamate molecule [1]. Significant research has been carried out in the past to utilize biotechnological solutions for the capture of CO_2, such as using the carbonic anhydrase enzyme [2]. Nevertheless, catalyzed desorption was only sporadically investigated. Amidases are a large group of enzymes that hydrolyze the C-N bonds found in a plethora of structurally diverse molecules. The sequence space for amidases is extremely large, with approximately 188,000 sequences present within the Amidase Signature superfamily alone. With this project we aim to identify highly efficient and stable amidase enzymes for low impact post-capture desorption of CO_2 from various carbamates, with special emphasis on hyperstable enzymes from extremophilic microorganisms due to harsh conditions (high pH and organic solvent concentrations) present in the process. We believe amidases carry untapped potential for enzyme-mediated CO_2 desorption at ambient temperatures.

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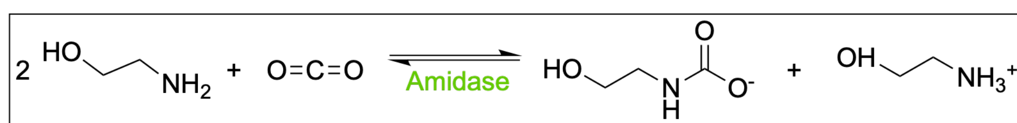

Figure 1 – Reaction diagram depicting the general idea behind the project. The carbamate molecule containing the captured CO_2 can be reacted with an amidase in the presence of an ethanolammonium ion to release the CO_2.

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A division of the extremophilic family GH57 into subfamilies

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In the CAZy database,[1] four GH families – GH13, GH57, GH119 and GH126 – have been considered the α-amylase families.[2] Among these, the family GH57 particularly attracted industrial attention as its characterized members are highly thermostable originated from thermophiles or hyperthermophiles.[3] Recently, GH57 was classified within novel clan GH-T with GH119,[4] confirming thus the original *in silico* prediction.[5] In 2018, a bioinformatics analysis of ~1,600 GH57 members emphasized eight distinct enzyme specificities forming their clusters in the phylogenetic tree.[6] The present study dealing with the updated dataset of ~5,000 sequences delivers the official division of the family GH57 into ten subfamilies. Each subfamily is highlighted by its sequence fingerprints, i.e. the logo of the five GH57 conserved sequence regions.[7,8] The structural features are also compared, with regard to diverse additional extra-domains and within the catalytic domain formed by the incomplete TIM-barrel with a succeeding α-helical bundle. Some interesting genomic contexts, not limited to Bacteroidetes PULs, are also illustrated. Confirming the previous study,[6] characterized members in each subfamily display a strong agreement in their functional profile, indicating that subfamilies improve the annotation power in GH57. Two subfamilies with so far only hypothetical proteins but conserved catalytic machineries deserve the attention, too.

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Structural determination of the polysaccharide portion of the catasan complex

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Biosurfactants are surface-active biomolecules produced by microorganisms with a wide range of applications. In recent years, due to their unique properties like specificity, low toxicity and relative ease of preparation, these surface-active biomolecules have attracted wide interest.[1]

Psychrobacter sp. TAE2020 is an aerobic γ-proteobacterium isolated from an Antarctic coastal seawater sample.[2] This marine bacterium can produce and secrete molecules endowed with surfactant and emulsifying properties. Furthermore, its cell-free supernatant strongly affected some specific virulence features of *Pseudomonas aeruginosa* isolates from CF patients.[2]

We recently isolated and purified the anti-adhesive and emulsifying polysaccharide-protein complex, CATASAN. The complex can reduce biofilm formation and the detachment of biofilm of the nosocomial bacterium *S. epidermidis*.[3]

Here we reported the purification and the characterization of the polysaccharide portion of CATASAN produced from *Psychrobacter* sp. TAE2020. The polysaccharide is associated with cells as a capsule. Finally, the polymer shows good adhesive properties on liposomes reproducing pulmonary surfactants.

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Biomining with salt and acid-loving extremophiles

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Iron- and/or sulfur- oxidizing acidophiles can be used in a process known as bioleaching in which they solubilize metals from insoluble critical minerals. However, most acidophilic microorganisms are sensitive to salt and, therefore, cannot be used in saline water biomining operations. Aside from osmotic stress, chloride ion toxicity is also a major challenge to acidophiles. This is because chloride ions can cross the cell membrane and disrupt the reverse transmembrane potential that these microorganisms maintain to survive acid stress. The entry of chloride ions into the cells causes acidification of the cytoplasm, resulting in cell death. The cost of desalinating process waters is a limiting factor to bioleaching in regions where high salinity exists in the ores and process waters. The best solution in such regions is the use of microorganisms that can tolerate both acid and salt stress. However, there are few naturally occurring environments where both acid and salinity stress exist simultaneously from which these microorganisms can be isolated. These include acidic salt lakes, acid mine drainage and volcanoes near seawater. The members of the genus *Acidihalobacter* are a novel group of microorganisms that have been isolated from these unique environments. Members of the genus *Acidihalobacter* are extremophilic microorganisms that are both halotolerant and acidophilic. They have been shown to release metals from insoluble mineral ores at up to 75 g/L sodium chloride stress at low pH (2-2.5) (1, 2). Our advanced bioinformatic studies have helped to phylogentically classify these microorganisms as well as to reveal interesting insights into their salt, acid and metal tolerance pathways, including the use of various transporters and channels (3-6). Pangenome analysis has confirmed that genes for halotolerance are conserved and stabilized (5). Proteomic studies of two of the species have further identified that the osmprotectant, ectoine, is the primary line of defence against high salinity (7, 8). However, genes for acid tolerance were found to have been obtained via horizontal gene transfer (9). Understanding the mechanisms and evolutionary adaptations of members of the *Acidihalobacter* genus can help direct future research towards their use in saline water bioleaching operations.

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Methylotrophic methanogenesis in thermophilic Korarchaeia, Methanomethylicia and Archaeoglobi revealed by cultivation, visualization, stable isotope tracing, genomics and transcriptomics.

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Archaeal mediated methanogenesis is the major source of methane, the second most abundant climate-active gas, and is thus critical for understanding Earth's climate dynamics. It was thought that methanogens were restricted to a few well-established groups within the phylum *Euryarchaeota*. Recently, environmental metagenomics and amplicon-based marker gene surveys have revealed the potential for diverse methanogenic pathways affiliated with multiple archaeal phyla. However, without cultivated representatives, laboratory experiments of predicted methanogens are restricted. Here, we cultivated two new methanogenic representatives of the phylum Thermoproteota, *Ca.* Methanodesulfokora washburnensis LCB3 (class *Korarchaeia*) and *Ca.* Methanosuratincola verstraetei LCB70 (class *Methanomethylicia*), as well as *Ca.* Methanoglobus hypatiae LCB24, a new methanogen in the class *Archaeoglobi* (phylum *Euryarchaeota*), from hot springs in Yellowstone National Park, WY, USA. Growth experiments combined with activity assays, stable isotope tracing, genomics and transcriptomics confirmed LCB3 and LCB70 grow via methyl-reducing hydrogenotrophic methanogenesis while LCB24 grows via methylotrophic methanogenesis on methylamines. The methyl-coenzyme M reductases (MCR) of all three cultures are highly expressed but are distantly related to canonical MCR, supporting the idea that methanogenesis is deeply rooted in the archaea. We also provide visulalization of LCB70's ultrastructure via cryo electron tomography, revealing structures related to motility, chemotaxis and cell-cell connections. The growth temperature of LCB3 (77 °C) also extends the upper temperature range of methyl-reducing methanogenesis. The cultivation of these archaea extends methanogenesis beyond the Euryarchaeota superphylum and is critical for a deeper understanding of the diversity, physiology and biochemistry of methanogens and their contributions to anaerobic carbon cycling in extreme environments.

Chemotrophic carbon fixation in the prony bay alkaline hydrothermal field

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Serpentinite-hosted hydrothermal vents count amongst the most extreme environments for life on Earth. Serpentinization, the geochemical alteration of mantle rock, produces a hyperalkaline milieu that curbs the cellular membrane potential and reduces the solubility of essential macronutrients. Despite these limitations, diverse microbial ecosystems develop in serpentinizing environments. They are most likely sustained by a community of hydrogenotrophic primary producers, whose metabolic functioning is essential to understanding modern serpentinite-hosted ecosystems and might provide insight into the origin of life[1].

One of the most important issues regarding primary production in a serpentinization context concerns the carbon source. As CO_2 precipitates at high pH, its bioavailability is drastically reduced. To date, four alternative carbon sources have been suggested: Bicarbonate, which can be redissolved from calcium carbonate by some microorganisms[2], as well as abiotically produced acetate[3], formate[4], and glycine[5]. Here, we present a metagenomic study on the uptake of those four potential carbon sources in the coastal serpentinite-hosted system of Prony Bay, New Caledonia[6]. Using custom HMM profiles built from the KEGG and MetaCyc databases, we investigate the presence of key uptake genes and carbon fixation pathways. This is performed for both binned and unbinned sequences from three sites along a transect from land to sea. We identify taxonomic groups playing a key role in primary carbon metabolism for each site and discuss implications for the metabolic functioning of the community.

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Diversity within species in South American terrestrial hydrothermal systems

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Hydrogeothermal ecosystems hold immense potential as model systems for the study of microbial ecology and evolution due to their uniqueness environmental factors and their biological and geochemical simplicity. In this work, we test the hypothesis that in these systems, in which steep environmental gradients are established, microdiversity compensates for the lack of general diversity. For this, we explored the occurrence, distribution, and microdiversity of two dominant prokaryotic taxa, the *Chloroflexia* and the *Acidithiobacillia*, at the two largest hydrogeothermal systems in South America, the El Tatio geyser field (ETGF) and the Copahue-Caviahue volcanic complex (CCVC) respectively. Using sequenced genomes from recovered isolates and genome resolved metagenomic techniques, we classified population-specific reads and evaluated the population structure of these taxa along temperature and/or pH gradients.

Distinct distribution patterns of each class representatives were observed, with differential abundance of specific lineages along the environmental gradients. Up to 10 lineages per class coexisted at specific sites, albeit with varying distributions in each case. *Chloroflexus/Roseiflexus* and *Fervidacidithiobacillus/Acidithiobacillus* partitioned along the temperature gradient in either system. Analysis of genomic indexes and single nucleotide variants profiling (SNVs) provided clear evidence of genetic differentiation within each lineage, correlating with specific environmental factors such as temperature at ETGF or pH CCVC. Results obtained indicate that local diversification of the dominant taxa occurs in both types of extreme environments and shed light on the ecological and evolutionary processes shaping microbial diversity in these ecosystems.

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Espresso solo con *s. Aci*? Growth of *Sulfolobus acidocaldarius* on spent coffee ground hydrolysates

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The industrial production of coffee beverages generates substantial side streams, including spent coffee grounds (SCGs), which annually contribute to about 6-8 million tons of waste [1]. However, this side stream contains valuable sugars, proteins, lipids, organic, as well as inorganic substances, which can be utilized for microbial growth [2]. Currently, they are often not utilized, leading to resource wastage and environmental concerns, such as landfill overloading and greenhouse gas emissions [4]. Isolating sugars from side streams often requires harsh processing conditions, such as acidic hydrolysis [5]. To subsequently cultivate mesophilic microorganisms, the extracts have to be adapted again, requiring high amounts of bases for neutralization [6], contradicting the sustainability.

I will show how we cultivate the thermoacidophile organism *Sulfolobus acidocaldarius*, using sugars extracted from various side streams. We explore its potential to utilize sugars derived from SCGs for producing value-added products such as highly stable lipids and carboxylesterases, the latter finding possible applications in plastic biodegradation. *S. acidocaldarius* takes up glucose, galactose, mannose, and arabinose, extracted from hydrolysates of SCGs. However, a balance between sugars and inhibitors, such as acetic acid, furfural, or 5-Hydroxymethylfurfural, which are co-extracted during the hydrolysis reaction, needs to be considered. Summarizing, I will show how to use the archaeon *S. acidocaldarius* in sustainability-driven processes.

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Guyparkeria halophila as a very efficient microbial platform for the circularization of carbon dioxide and thiosulfate into fine chemicals for the pharmaceutical industry

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The utilization of carbon dioxide (CO_2) emissions for valuable product production has emerged as a significant foundation for achieving a circular economy. However, current CO_2 conversion processes are still not cost-effective due to the use of a limited number of model microorganisms, the production of low-value compounds, and the requirement of cost-effective energy sources for CO_2 fixation. In this sense, if the elimination of CO_2 is developed simultaneously to the removal of industrial pollutant by-products, such as thiosulfate ($S_2O_3^{-2}$), the most abundant GHG would be cost-efficiently and sustainably eliminated. On top of that, if the simultaneous abatement of CO_2 and $S_2O_3^{-2}$ can produce fine chemicals, this process would promote the circularization of waste and the implementation of new bio-production systems. Ectoines are attractive compounds due to their high economic and social value. They are produced by prokaryotes at high salinity, and used as key ingredients for pharmaceuticals and cosmetics. This study targeted the transformation of CO_2 and $S_2O_3^{-2}$ into ectoines using unexplored bacteria. Firstly, genomic mining was conducted to find thiosulfate-oxidizing halophilic bacteria that contained the genes for ectoines synthesis. In total, 6 microbial genomes were identified as potential candidates to carry out the process. After laboratory validation of ectoine production at 3% NaCl, the fastest growing strain, *Guyparkeria halophila*, was selected for product optimization at three different salinities (6, 9 and 15% NaCl) using $S_2O_3^{-2}$ and CO_2 as the only substrates. Results showed that *G. halophila* accumulated significantly higher ectoine yields at 15% of NaCl. Finally, a batch bioconversion study, combining the optimal conditions previously determined was undertaken to observe ectoine production and contaminants depletion over time. Results showed that *G. halophila* reached maximum ectoine contents up to 47% (473.9 ± 37.1 mg of ectoine·g biomass^{-1}). These results not only constitute the highest ectoine yields so far reported by autotrophs and most of heterotrophs, but also, the first proof of a novel valorisation platform of CO_2 and $S_2O_3^{-2}$ that lay the foundation for a new economic niche aimed at CO_2 transformation into pharmaceuticals.

Gene Expression Under Stress Conditions in The Thermophilic PHA-Producing Bacterium *Caldimonas thermodepolymerans*

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Caldimonas thermodepolymerans is a moderate thermophile (45°C-55°C) that has shown the ability to produce polyhydroxyalkanoates (PHAs). It is studied for its potential as an industrial-scale bioproduction host. Its ability to produce poly(hydroxy-3-butyrate) by utilizing the monosaccharide xylose as a sole carbon source makes it a good candidate for agro-industrial waste valorization[1]. While polymer production is constitutive in this extremophile, the PHA yield increases under stress conditions. Therefore, in this work we aim to study the impact of nutritional limitations and stress conditions on the physiology and gene expression in *C. thermodepolymerans*. To this end, RNA-sequencing was performed for different conditions, supported by RT-qPCR analysis. Additionally, the amount of polymer formed inside the extremophile's cell was determined through Nile red assay and two-phase extraction[2]. The stress was shown to not only affect PHAs production but also impact other cellular functions. For high salinity, PHA production is enhanced while motility ability is diminished, and the flagella-related genes *flg*, *mot* and *fli* are downregulated under salt stress. This decrease in motility was monitored both on gene expression level and phenotypic level. In addition, the cells tested positive for biofilm production under different stress conditions. Also, an upregulation was observed for the genes responsible for secretion system 6 (T6SS), which are linked to biofilm formation[3]. Another gene of interest was *degU* encoding a putative global stress regulator. Several *degU*-like genes were identified in *C. thermodepolymerans,* and their regulation was screened under different conditions[4].

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Bacillus altitudinus sp. OQ983889- a novel halotolerant, soil fertility enhancer and heavy metal reducer isolated from the indian sundarbans

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Halotolerant microorganisms including bacteria, can grow in both high concentration of salt and also without salt. [1] There are several reports of halophilic/ halotolerant bacteria that are capable of functions like sulphur oxidization, phosphate solubilisation, cellulose degradation, production of antibiotics and enzymes etc [2]. In the current study, we discuss the beneficial aspects of a novel bacterial strain of *Bacillus altitudinus*, isolated from the soils of Indian Sundarbans. Primarily found to survive over a salt range of 0-20%; this bacterial strain was found to be highly tolerant towards Chromium (>2500ppm), Lead(>500ppm) and Nickel(>200ppm) and was able to reduce them significantly in broth culture. The percentage of reduction was check through MP-AES and mechanism was confirmed through SEM-EDX imaging. Further analysis also revealed exopolysachharide formation by the bacterial strain in presence of heavy metals. Additionally, the bacterial strain showed abiltiies for nitrogen fixation, phosphate solubilization and potassium solubilization. It could solubilize phosphorus and potassium as well produce ammonia in presence of low(1%) to a high salt concentration of 10%. Therefore, this novel strain, could be used as a possible solution for land reclamation in saline areas, minning areas as well formerly ill-used agricultural lands.

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From deep-sea volcanoes to human pathogens: evolutionary adaptations in Campylobacterota

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Deep-sea hydrothermal vents are permanently dark extreme environments primarily sustained by chemolithoautotrophic microorganisms. Campylobacterota (formerly Epsilonproteobacteria) are the pioneer colonizers of newly formed vents and establish biofilms along the steep physical and redox gradients characteristic of these environments.

Besides their role as primary producers at deep-sea hydrothermal vents, Campylobacterota established symbiotic and/or pathogenic relationships with animals, which led to interactions with other symbiotic microorganisms, potentially leading to gene exchange. To date, horizontal gene transfer has been proposed as the principal explanation for the acquisition of virulence genes in pathogenic Campylobacterota. We posit that Campylobacterota originated in volcanic habitats and later diversified to colonize more 'recent' ecosystems, like the gastrointestinal tract of mammals. Since Campylobacterota includes human pathogens, such as Campylobacter jejuni and Helicobacter pylori, they provide excellent models to investigate the emergence and evolution of pathogenicity. In this study, we carried out comparative genomic analysis to identify the shared and lost/acquired genes of 36 Campylobacterota strains adapted to different environmental conditions (deep-sea vents, sulfidic habitats, and the gastrointestinal tract of mammals). Understanding the gene flow within the Campylobacterota will allow us to trace the evolutionary trajectory of this group of bacteria from deep-sea volcanoes to commensal of mammals and human pathogens.

Biological and Non-biogenic Filamentous Mineralization

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Abiotic precipitation typically conforms to the formation of geometric shapes. This is seen in the many small crystals from the fast nucleation in supersaturated solutions to the large faceted structures from the slower growth in more dilute environments. Across these nano- to macrometer length scales, the morphological traits of flat faces and sharp edges persists from the self-assembly of either atoms or of larger nanoparticles and has guided the prevailing view of precipitation in the absence of life. The near equilibrium conditions for these crystallizing systems are in stark contrast to the products forming in complex physicochemical conditions within far-from-equilibrium settings. Under such environments, surprising structures and patterns emerge for both biological and abiotic systems. Abiotic examples include the long-standing laboratory experiment producing hollow chemical gardens, the spatial organization of Liesegang rings, and, more recently, the biomimetic precipitation of biomorphs. Approaching the physics and chemistry governing from biological and abiotic precipitation will provides a deeper understanding for how and why both systems are able to achieve similar results and inform the analysis of naturally occurring examples on Earth and other planetary bodies.

Cold adaptations in bacterial symbionts from antarctic sponges: what differentiates host-related and free-living bacteria?

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Marine sponges harbor abundant and diverse microbiomes essential for maintaining the health and development of the host[1, 2]. Sponge holobionts, the assembly of sponges and their microbiome, are ecosystem engineers in benthic environments worldwide[1, 3]. Antarctica is a harsh and geographically isolated environment where sponge holobionts dominate[4]. However, very little is known about the sponge holobiont adaptation to cold conditions and how the microbiome contributes to it. Here, we explored bacterial functions for cold adaptation in Antarctic sponge-associated microorganisms and compared their distribution against sponges from other environments and the surrounding seawater. To do so, we processed sponge metagenomes from Antarctic, tropical, and temperate environments and metagenome-assembled genomes of Antarctic sponges and seawater. Antarctic sponge microbiomes significantly exceeded the proportion of genes codifying bacterial Cold shock proteins (Csp) and Osmoprotectant-related functions (Osm) compared to tropical and temperate sponges. More than half of the total csp and osm genes belonged exclusively to Antarctic sponge symbionts. Moreover, orthologs of the cspC and cspG genes were present in most of the Antarctic sponges, while practically absent in the seawater. Genomic analysis revealed the presence of the Type VI Secretion System in Antarctic sponge symbionts, and CspC and CspG correspond to known effectors of this symbiotic mechanism. Overall, these results suggest that proteins for cold adaptation could be exported from the symbiont bacteria to the sponge host through this secretion system.

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Extremophiles of contaminated Environments: surviving harsh metabolic and toxic settings

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Everywhere and anywhere, we find microbes surviving beyond limits of life in all the multiple facets possible[1,2]. Natural and anthropogenic environmental pollution acknowledgeably has contributed to unprecedent discharge of both natural and synthetic pollutants beyond what is acceptable for survival of most organisms. Microbes surviving in environments with extreme and diverse pollution levels have learnt to thrive in all physiological, metabolic, genomic sense among the rest, to be able to be residents of such habitats[1,2]. Microorganisms surviving in environments of extreme pollution for example of metal, pesticide petrochemicals, radioactive have been described and bioprospecting of such and more continue to be key in revolutionising microbial diversity discoveries, biotechnology and industrial applications. Ideal microorganisms with polyextremophilic characteristics have the added advantage of being able to adapt to both their natural extreme conditions in addition extreme pollutant challenges. In this paper key highlights on diversity, bioprospection and recent developments in microbiology of extreme polluted environments are described.

Keywords:
Polyextremophilic, anthropogenic environmental pollution, bioprospection, diversity

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Towards the establishment of thermophilic methanol-based microbial cell factories: rewiring the metabolism of *p. Thermoglucosidasius*

for methanol dependency

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Microbial cell factories are a sustainable approach for the biobased production of chemicals at an industrial scale, applying microbial hosts to convert feedstocks into valuable compounds. Typically, sugar-based feedstocks derived from crops, such as glucose or molasses, are used for biobased production[1] . However, given sustainability and scalability concerns on using sugar from agriculture for bioproduction, widening the feedstock range for microbial cell factories is of high interest. Among several alternatives, methanol is a one-carbon alcohol that stands out as a feedstock for the bioproduction of chemicals. Methanol can be used as a sole carbon and energy source by certain microorganisms. It is a soluble feedstock, electron rich, and can be sustainably produced from organic waste (bio-methanol) or from CO2 and renewable electricity (e-methanol). These characteristics make methanol an ideal feedstock for the bioproduction of chemicals[2] . *Parageobacillus thermoglucosidasius*, although non-methylotroph, is a thermophilic bacterium, which is emerging as one of the most promising thermophilic microbial hosts for the bioproduction of chemicals[2] . Here, we report on how we are converting *P. thermoglucosidasius* into a methanol-assimilating organism through the synthetic rewiring of its native metabolism

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Screening for pahs-degrading bacteria from oil-contaminated saline soil and using real time pcr to assess changes in catechol 2, 3-dioxygenase and *alkb* gene copy numbers

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In this study, we conducted an investigation into the isolating and characterizing hydrocarbon-degrading bacteria capable of degrading PAHs such as anthracene, naphthalene, and pyrene. First, we collected samples from marginal soils around the evaporation ponds in south oil fields in Iran. Next, we isolated and screened bacterial strains from the contaminated soil samples using Bushnell Haas medium supplemented with crude oil as sole carbon sources. Three bacterial isolates, designated as strains PAB37, PAB310, and PAB314 were selected based on their ability to degrade PAH compounds (naphthalene, anthracene, and pyrene) efficiently. They are able to degrade 70 mg/l of each PAH compounds (naphthalene, anthracene, and pyrene) within 30 days. According to the results from 16S rRNA gene sequencing, the strains PAB37, PAB310, and PAB314 with high sequence similarity (> 98%) were tentatively identified as *Idiomarina zobellii* PAB37 (PP358161), *Dietzia maris* PAB310 (PP358162), and *Planococcus antioxidans* PAB314 (PP358163) respectively. Real-time polymerase chain reaction method to quantify the proportion of microorganisms containing alkane monooxygenase and catechol 2,3-dioxygenase used to monitor changes in the microbial community in oil-contaminated soil samples compared to the uncontaminated soils. Our results demonstrated a significant upregulation of *alkB* and catechol dioxygenase genes in the polluted soil samples, indicating enhanced number of petroleum-degrading bacteria.

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Leveraging extremophiles for advanced bioprocesses in Europe through Industrial Biotechnology and the IBISBA Research Infrastructure

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The circular bioeconomy relies on renewable biological resources to drive resource-efficient processes, combining economic strength with environmental sustainability. However, to bolster the circular bioeconomy's competitiveness, research and innovation (R&D) that upholds sustainable solutions is imperative. In this regard, industrial biotechnology (IB) emerges as a transformative force equipped to compete with fossil-dependent industries. Leveraging extremophilic microorganisms and enzymes, IB transforms biomass into products across sectors such as energy, agriculture, forestry, and pharmaceuticals. Europe's research landscape is a crucial asset for IB R&D, spanning the lab to pilot scale and entire bioprocess development pipeline. However, a fragmented landscape poses challenges, hindering knowledge, consolidation and impeding true end-to-end bioprocess development[1]. In this context, IBISBA, a unique pan-European distributed research infrastructure dedicated to IB, strives to overcome these hurdles, fostering knowledge sharing and best practices, and accelerating R&D[2].

IBISBA federates European expertise from 23 countries (nodes) to provide researchers from academia and industry worldwide with integrated services for all stages of bioprocess development projects. IBISBA facilitates accessibility to national research facilities and services across Europe. Each facility contributes its unique competence, forming the links in the biotechnology R&D chain. The Italian node IBISBA-IT plays a significant role in developing new bioprocesses through the discovery and engineering of carbohydrate-active enzymes from extremophiles.

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Valorization of biomasses from energy crops for the discovery of novel thermophilic glycoside hydrolases through metagenomic analysis

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The growing interest in environmentally friendly technologies drives the transition from a fossil-based economy to a bioeconomy. A key factor in enabling a circular bioeconomy is the valorization of renewable biomasses as feedstock to extract high value-added chemicals. In this transition, the discovery and use of robust biocatalysts to replace toxic chemical catalysts play a significant role as technological drivers. To meet these demands, we performed microbial enrichments on two energy crops, used as low-cost feed for extremophilic consortia. A culture-dependent approach combined with metagenomic analysis led to the discovery of more than 300 glycoside hydrolases and the characterization of a new α-glucosidase from an unknown hyperthermophilic archaeon. Aglu1 proved to be the most active archaeal GH31 on 4Np-α-Glc and showed unexpected specificity towards kojibiose, making it a promising candidate for biotechnological applications such as the liquefaction/saccharification of starch[1].

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A Novel Thermostable Enzymatic Cocktail from Extremophilic Microorganisms for Transforming Carbohydrates in Lignocellulosic Waste Biomass

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In a circular economy context, targeted degradation of polysaccharides in lignocellulosic waste biomasses by an enzymatic cocktail of thermostable carbohydrate active enzymes from extremophilic microorganisms is a valuable strategy for producing high-added value saccharides. Among these biomasses, Spent Coffee Grounds (SCG), composed of galactomannan, arabinogalactan II, and cellulose [1], stand out as an excellent source of high-value saccharides that are well-suited for bioethanol production, fermentation, synthesis of biodegradable polymers and materials, and other high-value products [2]. In this context, (hyper)thermostable and thermoactive (hemi)cellulases, due to their stability, play a pivotal role in the saccharification of recalcitrant polysaccharides and oligosaccharides under harsh industrial conditions [3]. In this study, we effectively selected thermophilic and thermostable enzymes based on pH, thermal stability, and activity to set up an enzymatic cocktail for the hydrolysis of SCG. Various mild delignification pretreatments were applied to the raw SCG, resulting in SCG-derived biomass with reduced lignin content and enhanced accessibility for enzymatic hydrolysis. A mix of thermostable endo β-mannanase and α-galactosidase is used to produce a significant amount of mannooligosaccharides (MOSs). These MOSs significantly enhanced the growth of probiotic bacterial strains and biofilm formation, suggesting their potential as prebiotics. Furthermore, setting up a new enzymatic cocktail of eight different GHs, which successfully transformed 52% of the carbohydrate content of microwave-pretreated SCG into oligo and monosaccharides that could be used in multiple applications.

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Sustainable nanotechnology: silver nanoparticle production using secretomes of geobacillus stereothermophilu gf16

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Extremophiles have long been recognized for their extraordinary potential in various applications. These microorganisms are particularly valuable due to their ability to produce a wide range of robust enzymes and bioactive molecules. The bacterial secretome plays a crucial role in various biological processes, including cell-to-cell communication and environmental interactions. Additionally, these secreted proteins offer significant potential as sources of enzymes and bioactive molecules for biotechnological applications, such as the production of nanoparticles[1].
This study explores the biosynthesis of silver nanoparticles (AgNPs) using cell-free secretomes from Geobacillus stearothermophilus GF16, a thermophilic bacterium isolated from a thermal spring in Pisciarelli (Naples)[2]. AgNPs are renowned for their distinctive physical and chemical properties, making them applicable across diverse fields. Traditional physical and chemical methods for synthesizing AgNPs are environmentally detrimental and generate substantial side products. Hence, there is a growing interest in alternative, eco-friendly synthesis methods.
The biosynthesized AgNPs were characterized using UV–Vis spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS) and exhibited interesting biological activities. These findings position Geobacillus stearothermophilus GF16 as a versatile producer of high-value molecules. Its secretome demonstrates significant potential for eco-friendly synthesis of AgNPs. Leveraging the unique capabilities of extremophilic microorganisms like GF16 can lead to greener biotechnological processes.

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Genomic mining of *Geobacillus stearothermophilus* gf16 for xylose production from hemicellulose-rich biomasses using secreted enzymes

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The valorization of lignocellulosic biomass, derived from various bio-waste materials, has received considerable attention as a sustainable approach to improve production chains while reducing environmental impact. Microbial enzymes are known to be key players in the degradation of polysaccharides, offering versatile applications in biotechnology and industry; among them, glycoside hydrolases (GHs) play a central role. In particular, xylanases are used in a wide range of applications and are essential for the production of xylose, which can be fermented into bioethanol or find use in many other industries. Currently, fungal secretomes dominate as the main reservoir of lignocellulolytic enzymes, but thermophilic microorganisms offer notable advantages[1]. *Geobacillus stearothermophilus* GF16 is an extremophilic bacterium isolated from the solfataric mud pool in Pisciarelli, a well-known hydrothermally active zone of the Campi Flegrei volcano located near Naples; its genomic characterization has revealed genes encoding putative enzymes involved in lignocellulose degradation. We analysed the thermostable GHs secreted and found them active on different natural polysaccharides and synthetic substrates, confirming the presence of an array of inducible GH activities. In particular, the concentrated secretome possesses significant thermostable xylanase and β-xylosidase activities highlighting its potential for application in biomass valorization. Indeed, the concentrated secretome of the strain cultivated on xylan showed hemicellulose hydrolysis capabilities on various agri-food wastes and produced xylose with a 300-fold increase in release compared to a commercially available cocktail[2]. This study expands the understanding of lignocellulose-degrading biocatalytic systems in thermophilic bacteria and offers a promising approach for sustainable xylose production from waste biomasses.

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Thermophlic biocatalysts for agri-food waste valorization into functional bioproducts

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The valorization of agrifood residues (AFR) for the green production of biomaterials, is a hot topic in the circular economy concept. Among AFR, citrus residues have been successfully used as carbon sources in culture media for microbial fermentation[1] although harsh chemical pretreatments have been used with high environmental impact [2].

In order to establish an ecofriendly and sustainable strategy for the production of lactic acid (LA), a moderately thermophilic LA producer *Weizmannia coagulans* [3] strain was employed, by exploiting its degradative ability on complex biomasses [4].

Our result show: i) the feasibility of replacing the standard growth medium with less expensive carbon source which is widely available at lowcost, and suitable for fermentation growth and ii) the setting up of a green process for LA production based on untreated biomass.

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Acidiphilium acidophilum CJR1, an Arsenite-oxidizing Autotrophic Acidophile: Physiology and the Transcriptomic Response to As(III)

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In previous investigations to treat acidic As(III)-containing solutions, an autotrophic acidophilic arsenite oxidizer, *Acidiphilium acidophilum* CJR1, has been isolated from an acid mine-drainage puddle in the abandoned silver mine Reiche Zeche in Freiberg, Germany. This strain grows at pH 2 with As(III) as sole electron donor and just CO_2 from air as carbon source since more than three years. However, it grows faster and oxidizes As(III) faster in the presence of 0.02% yeast extract than under strictly autotrophic conditions. Interestingly, the stain neither oxidizes iron nor elementary sulfur, thiosulfate, or tetrathionate. Genome sequencing revealed a greater size (5.16 Mbp) than found in other *Acidiphilium* strains. Of the 11 contigs obtained, 4 obviously are circular plasmids. Four gene clusters with As(III) resistance genes were found of which one also comprises genes of a presumed arsenite oxidase. To obtain some insight into the physiological response of strain CJR1 towards As(III), an RNA-Seq approach was employed. Though capable of autotrophic growth with arsenite, due to considerably better growth in the presence of yeast extract, *A. acidophilum* CJR1 was cultured under two heterotrophic conditions, one with additional arsenite and, as a control, one with yeast extract and 20 µM Fe^{2+}. The results indicated the expression of arsenite oxidase genes under both conditions, suggesting that the enzyme may be constitutively expressed. In the As(III) condition, the COG categorization indicated that the most strongly upregulated genes were energy-related or chaperones. These results provide the first transcriptomic insight into the arsenite response of an *Acidiphilium acidophilum* strain.

POSTER ABSTRACTS

Conversion of industrial wastewater nitrogen compounds to ammonium by microaerobic activated sludge system for circular bioeconomy

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Nitrogen compounds in wastewater are currently removed by microbial nitrification and denitrification in the energy intensive activated sludge system. Meanwhile, there are increasing interests in the nitrogen recycling as resources, such as raw material and fuel. Recently, microaerobic activated sludge system has been proposed to convert nitrogen compounds in wastewater to ammonium [1]. The further combination with the membrane concentration and separation technologies enables the recovery of the highly concentrated ammonia solution. In the biological system, it is essential to suppress microbial nitrification under low aeration conditions for ammonium retention, while removing organic carbon components almost completely. The sludge microorganisms are forced to degrade high concentrations of the organic components with the minimal requirement of nitrogen source. In this study, with a focus on the lab-scale microaerobic activated sludge system treating an acidic wastewater from a fermentation industry, we first investigated the effective neutralizer (slaked lime or NaOH) to minimize metal elements that caused membrane fouling in the following process, and then examined the availability of excessive waste sludge for ammonium yield in the microbial conversion. The system performances were evaluated by tracking the total organic carbon, total nitrogen and NH_4^+ concentrations, while residual dissolved components in the treated wastewater were characterized by ICP-MS-MS and LC-TOF-MS. The prokaryotic and eukaryotic microbial communities were assessed by 16S and 18S rRNA gene sequencing. In addition, high-sensitivity stable isotope probing of rRNA [2] was applied to identify the sludge microorganisms capable of degrading the excessive waste sludge material (i.e., microbial biomass) for ammonium yield. The detailed results will be given and discussed in the presentation.

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A novel endo-1,4-β-xylanase from alicyclobacillus mali fl18: biochemical characterization and its synergistic action with β-xylosidase in hemicellulose deconstruction

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A novel endo-1,4-β-xylanase-encoding gene was identified in *Alicyclobacillus mali* FL18 and the recombinant protein, named *Am*Xyn, was purified and characterized [1]. The monomeric enzyme exhibited an optimal activity at pH 6.6 and 80 °C and a good catalytic efficiency on beechwood xylan, compared to other thermophilic xylanases. In addition, the enzyme did not display any activity on cellulose, suggesting a possible application in paper biobleaching processes. To develop a new enzymatic thermophilic cocktail for xylan degradation, the homosynergic association between *Am*Xyn and the previously characterized β-xylosidase *Am*βXyl [2] was assessed. The highest degree of synergy was obtained when *Am*Xyn and *Am*βXyl were added sequentially on beechwood xylan. This cocktail was also employed for the hydrolysis of wheat bran residues and the sugar content was evaluated by TLC and HPAED-PAD. The high amount of xylose released suggested their potential application as promising biocatalysts in the saccharification of agricultural wastes.

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Use of different culture dependent strategies for screening of the antibacterial potential of moderately halophilic bacteria isolated from Provadia salt deposit

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In the 21st century, human society faces a new global health problem - antibiotic resistance. As a consequence, the antibiotics traditionally used today are becoming less effective. This necessitates the search for new antimicrobial compounds (AMC). Particular research interest has been focused on previously underinvestigated natural sources. Extremophilic microorganisms and in particular halophilic bacteria living in saline and hypersaline habitats are a poorly studied but inexhaustible source of diverse secondary metabolites including AMC.

The aim of our study was to isolate halophilic bacterial producers of AMC from Provadia salt deposit in Bulgaria.

Initially an optimisation of the isolation procedure conditions was made in order to achieve a maximal recovery rate of bacterial species. Then, moderately halophilic bacterial strains were isolated from saline soil samples collected from the field of Provadia salt deposit. At the primary screening their antibacterial activity was evaluated by the agar overlay assay against *Escherichia coli* and *Staphylococcus epidermidis*. The halophilic bacterial isolates demonstrated activity were subjected to a secondary screening against ESKAPE-group safe relatives by agar disk diffusion and agar well diffusion assays.

The optimal isolation procedure involved soil suspension in 50 mM sodium pyrophosphate, 3 min vortex mixing and cultivation on HM 10% NaCl medium[1]. At the primary screening 6 strains showed activity against *Staphylococcus epidermidis* and 12 strains against *Escherichia coli*. The conducted Waksman crowded plate assay resulted in the isolation of 37 active stains that were able to inhibit the growth of the surrounding bacterial colonies.

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Mutagenesis of a thermophilic hydrolase for increased activity at lower temperature

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The rigidity of a protein has been extensively linked to its thermostability [1-3]. This is reflected in the sequences of such proteins in that thermophilic proteins tend towards having shorter chains than psychrophilic enzymes from the same class [4], and more thermophilic proteins tend to have shorter 'loops' connecting secondary structure elements [5].
For enzymes to be useful industrially, high specific activity combined with thermal stability are desirable features. Additionally, the alteration of substrate specificity to better match the desired industrial use is ideal.
In this work, a thermophilic hydrolase was mutated by rational mutagenesis, and extensions to loops with varying lengths were inserted. The aim was to increase the activity of the enzyme at low temperatures, while maintaining the stable framework of a protein from a thermophilic organism.
3 mutants of the original hydrolase were produced and successfully expressed in *E. coli*. Of these mutants, one (mutant 2) displayed increased specific activity at room temperature against the optimum substrate, while maintaining comparable thermal stability. All three mutants displayed increased activity against a wider range of substrates, with mutant 5 showing an optimum substrate with a different carbon chain length. While mutant 3 demonstrated reasonable residual activity after storage at 37°C for 4 weeks, the other mutants showed significantly reduced or eliminated activity after this time. Therefore, further development of these mutants is required to make them suitable for industrial use.
This work demonstrates that the thermal stability of a protein from a thermophile can be maintained while improving the activity for industrial use. Further mutations to stabilise the variants further may increase the storage stability, as well as by optimisation of storage conditions.

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Extremophilic conversion of syngas into valuable osmolytes

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The use of syngas from the gasification of organic waste appears as a potential substrate for the development of a novel bioindustry that enforces pollution control and secures a circular and climate neutral economy. However, the biotechnological valorization of syngas into chemicals is still restricted by the limited number of model microorganisms implemented, and the small profit margin of the products synthesized. Ectoine and hydroxyectoine (HE) can be produced by microorganisms growing at high salinity as osmoprotectants. They are high-value chemicals for the pharmaceutical and medical sectors (1000–1200 € kg^{-1}). Ectoine is currently produced in the bio-industry via sugar fermentation with high production costs, while HE industrial production is not yet cost-effective. In this work, genome mining was used to find microorganisms capable of converting carbon monoxide (CO) from syngas into these fine chemicals. The aerobes *Hydrogenibacillus schlegelii*, *Hahella chejuensis*, *Alkalispirillum mobile*, *Mycobacterium smegmatis*, and *Alkalilimnicola erlichii* were detected as putatively able to transform syngas into both osmolytes. During laboratory validation we demonstrated the capability of *H. schlegelii* to produce HE using syngas as the sole substrate. Interestingly, ectoine was not detected and HE was obtained as an isolate product. Thus, according to these results, the use of a thermophilic and autotrophic bacteria can be a good approach to produce HE as the sole osmoprotectant without the use of genetically modified bacteria. Further optimization of salt, temperature, and CO/H_2 ratio in batch bioreactors showed that at 5% NaCl, this bacterium yielded HE at 46.70 ± 2.8 mg g·$biomass^{-1}$. Deviations from the optimal temperature negatively impacted bacterial growth and consumption. Moreover, the concentration of CO/H_2 in the gas phase proved to be crucial. Specifically, when the gas composition contained 70% CO and 10% H_2, the average specific HE was 2 x times higher (57.0 ± 5.0 mg g·$biomass^{-1}$) compared to when it contained 30% CO and 25% H_2. Shotgun genomics analysis revealed that aerobic carbon monoxide (CO) growth was possible at high salinity with the presence of the genes *coxS*, *coxM*, and *coxL*, as well as the genes for CO_2 fixation (RuBisCo), providing deeper insights into CO aerobic metabolism, which has been poorly understood thus far.

Low Nitrous Oxide Emission by Comammox Bacterium *Nitrospira inopinata* under Acidification

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Ammonia-oxidizing microorganisms (AOM), which include both ammonia-oxidizing bacteria (AOB) and archaea (AOA), play a crucial role in the nitrogen cycle and significantly influence the global production of nitrogen oxides, such as N_2O and NO. The mechanisms driving the production of these gases, both biological and non-biological, vary among these microbial groups. Recent studies on nitrogenous gas compound production pathways in complete ammonia oxidizers (Comammox) have expanded our understanding.
This research provides new insights into NO and N_2O production from the Comammox bacterium, *Nitrospira inopinata*, under different pH conditions. We found that N_2O production was lower at acidic pH levels compared to neutral pH, a behavior that contrasts sharply with other AOMs, which do not show reduced N_2O production at lower pH levels. This result was corroborated by isotopic ratio mass spectrometry (IRMS) data. The reduction in N_2O production at low pH was linked to lower concentrations of NH_2OH, suggesting a possible role of abiotic NH_2OH decomposition. Additionally, transcriptomic analysis revealed variations in enzyme expression between acidic and neutral pH conditions, shedding light on the processes contributing to the N_2O production pathway. These findings, along with comparisons to other AOMs, enhance our understanding of nitrogen cycle dynamics and the complex processes influencing greenhouse gas production by AOMs. The significance of this research is underscored by shifting environmental conditions, particularly the phenomenon of global acidification.

Exploring hypersaline red brine from a salt-pan for halophiles producing polyextremophilic alginate lyases with antibiofilm activity

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Hypersaline habitats, like salt pans near salt lakes, harbour novel microbes bearing polyextremophilic macromolecules, particularly enzymes. The significance of alginate lyase to the pharmaceutical industry necessitates its mass production and purification. The major bacterial sources of lyases viz. *Azotobacter*, *Vibrio*, *Microbulbifer*, and *Bacillus* spp. from marine habitats are feebly stable, which renders them less useful. This leads to investigation of halophilic microbes producing alginate lyases with high stability under extreme conditions. Interestingly, the alginate lyases inhibit biofilm formation in pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Hence, these can be used in antimicrobial drug preparations. In this study, a *Bacillus licheniformis* was isolated from a hypersaline red brine of a salt pan near Sambhar Lake, Rajasthan, India. The isolation used minimal media agar plates with varying salt (NaCl) concentrations (5, 10, 15, and 20% w/v), yielding 13, 6, 5, and 3 bacterial colonies, respectively. These were picked and purified using the quadrant streaking method, repeated several times, and ensured through light microscopy. Alginate lyase activity was checked on alginate minimal media-agar plates, showing clear zones around active colonies. Notably, four colonies from 5% NaCl and two from 10% NaCl showed lyase activity, while those from higher NaCl concentrations did not. The assessment of the active isolates was performed to quantify the lyase production, which was evaluated as 1.12, 1.61, 1.95, and 3.81 IU/mL in 5% while 0.23 and 0.51 IU/mL for 10% NaCl grown isolates, respectively under optimized conditions obtained from OFAT analysis. Hence, an isolate EMB42 with the highest alginate lyase production (3.81 IU/mL) was selected and identified through the 16s rRNA technique as *Bacillus licheniformis*. Furthermore, the zymography analysis of the partially purified enzyme solution showed an extracellular lyase (42kDa) produced by EMB42. The enzyme preparation was investigated for pH and temperature optima, 8.0 and 45 °C, respectively. The lyase exhibited high substrate specificity towards alginate, polyG, and polyM. Further, the enzyme preparation of 0.1 IU showed a 58.42% biofilm inhibition rate for *P. aeruginosa*.

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Differential growth response of hot desert and polar desert hypolithic cyanobacteria to thermal stress

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Desert cyanobacteria have evolved to thrive under extreme temperatures and water stress in both hot and cold climates, where they play a vital role as important primary producers. Climate change is predicted to severely affect desert environments relative to less arid climates, causing major changes in temperature and water availability. To explore the potential response of desert cyanobacteria to climate change we performed a series of growth experiments. Cultures were isolated from hypolithic biofilms recovered from two hyper-arid regions: the Atacama Desert in Chile (hot desert) and the McMurdo Dry Valleys in Antarctica (polar desert). For each desert, we isolated two representative cyanobacterial taxa, *Chroococcidiopsis* sp. and *Phormidium* sp., which are among the most abundant taxa recorded in desert hypolithic biofilms. Cyanobacteria growth response was tested against a range of temperatures from 10 – 30 °C to simulate current and future growth conditions. *Phormidium* sp. from the hot and polar deserts grew strongly at all temperatures between 15 – 30 °C and shared an optimal growth temperature of 20 °C while no significant growth was recorded for either strain at 10 °C. *Chroococcidiopsis* sp. from the hot desert displayed strong growth in the range of 15 – 30 °C, with optimal growth at 30oC. In contrast, the polar strain grew strongly within a narrower range of 10 – 20 °C, with an optimum 15 °C and drastically reduced growth at higher temperatures. Based on these findings, it is hypothesized that, subject to moisture availability, hot desert cyanobacterial growth may be resilient to warming temperatures within the 5 – 7 °C maximum of climate predictions for deserts. The results also suggest that a significantly warmer Antarctic may lead to a shift in dominance towards *Phormidium* sp., which demonstrated greater thermotolerance than *Chroococcidiopsis* sp. Ongoing research is seeking to further resolve the relationship between thermal and xeric stress on the growth of hypolithic desert cyanobacteria.

Taming of l-asparaginase from hyperthermophile *Thermococcus sibiricus* for biotechnology application

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L-asparaginase (L-ASNase) is a vital enzyme that hydrolyzes L-asparagine to L-aspartic acid and ammonia. The enzyme is known as a mainstay of blood cancer treatment. In the food industry, L-ASNase prevents the formation of carcinogenic acrylamide in foods processed at temperatures above 120°C. Among the various organisms expressing L-ASNases, hyperthermophiles produce enzymes with superior properties. Previously, after codon optimization, a stable, highly active at 90°C L-ASNase from the hyperthermophilic archaea *Thermococcus sibiricus* (TsAI) was expressed in *Escherichia coli*.

In this study, an attempt to improve the properties of the wild-type enzyme TsAI using protein engineering was performed. A total of 10 mutants with single or multipoint substitutions of highly conserved amino acid residues of thermophilic, but not mesophilic L-ASNases ("special residues"), were obtained. The enzymes were successfully expressed in *E.coli* BL21 (DE3). Based on the results of screening the enzymatic activity of crude extracts at 24-90°C, 6 mutants displaying high specific activity were purified by ion-exchange chromatography. Comparative studies revealed that substitutions of so called thermophilic "special residues" affect the optimum temperature, specific activity, substrate specificity and thermostability of the enzyme.

Among the obtained variants, two mutants TsA293 and TsA_gsq displayed more than 2-fold increase in activity compared to the wild type enzyme at the optimum temperature of 90 °C. Despite a slight decrease in thermostability, both enzymes are promising for high-temperature food technologies.

From the group of TsA forms with a lower optimum temperature, 4 mutant exhibited increased activity at 37 °C compared to the wild type enzyme, including glutaminase-free TsA112. Due to high activity and stability, these variants have potential for biomedical application.

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Metabolic and genomic study of a new hyper-alkaliphilic hydrogen-utilizing bacteria isolated from the prony hydrothermal field; description of *Serpentinimonas pronyense* sp. Nov.

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A novel hyper-alkaliphilic bacterium, designated strain SMBJO2^T, was isolated from the Prony Hydrothermal Field (New Caledonia)[1,2]. Cells are thin, motile, Gram-negative flexible rods. Strain SMBJO2^T grows at temperatures of 30-37 °C, pH 10-11, and NaCl concentration between 0-2 g/L. 16S rRNA gene sequence shows that *Serpentinimonas maccroryi* str. B1^T, *Serpentinimonas raichei* str. A1^T and *Serpentinimonas barnesii* str. H1^T are the closest relatives[3,4] (99.2%, 98.0 % and 97.3 % 16S rRNA gene identity respectively). Strain SMBJO2^T is a member of the family *Comamonadaceae*, in the order *Burkholderiales* and class *Betaproteobacteria* of the phylum *Pseudomonadota*. The genome consists of a 2.6 Mb chromosome with a 67.3 % GC content. Strain SMBJO2^T showed a metabolic versatility. It grows chemoorganotrophically by utilizing proteinaceous substrates (yeast extract, tryptone), and organic acids (acetate). It can grow by anaerobic respiration of nitrate, as an electron acceptor, to N_2 (denitrification) or NH_4 (DNRA). Strain SMBJO2^T grows also chemolithoautotrophically using H_2 as an electron donor (hydrogenotrophy), and nitrate or O_2 as electron acceptors. Genome analysis suggests that strain SMBJO2^T performs autotrophic carbon fixation *via* the reductive citrate cycle. Genomic comparison of strain SMBJO2^T with *Serpentinimonas maccroryi* str. B1^T showed that ANIb was 91.94 % and dDDH was 64.1 %. The dominant cellular fatty acids were C18:1(11), C16:0, C18:0 and C16:1(9) (respectively 33.8, 20.0, 18.0 and 16.6 %). Based on genomic and phenotypic properties, strain SMBJO2^T is proposed to be the type strain of a novel species *Serpentinimonas pronyense*.

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PCR scanning-based genome trimming facilitates construction of *Saccharolobus islandicus* cells with minimized genomes

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Microbial genomes contain a large portion of non-essential genes that function in maintaining their ecological fitness but are dispensable from constructing chassis cells for biotech applications. Transposon inactivation experiments revealed that bacteria and archaea only carry a few hundred of essential genes.[1,3] However, further researches demonstrated many genes are conditionally essential and their identification often involves laborious trial-and-error experiments. We designed a novel CRISPR targeting-based strategy that greatly simplifies their identification. This involved a two-step process, including (a) CRISPR targeting-based PCR scanning to classify essential vs. nonessential gene blocks, and (b) genome trimming of identified nonessential gene blocks with essential genes as homology arms. The method was tested using *Saccharolobus islandicus*, a thermophilic acidophile,[2] which carries 6 predicted nonessential regions (N1 to N6).[3] CRISPR genome editing revealed only N4 is deletable, suggesting the remaining 5 regions carry unknown essential genes. To identify these new essential genes, N1 and N3 regions were divided into 4 and 8 gene blocks, for which homology arms (HAs) were designed. These HAs were tandemly arranged to yield synthetic genomic fragments that could replace the corresponding regions in N1 or N3 upon CRISPR genome editing. PCR scanning with HA primers identified nonessential synthetic segments, which were used to discern essential versus nonessential gene blocks. New essential genes were then predicted and tested by using their coding sequences as HAs. In the end, *S. islandicus* cells with reduced genome sizes were obtained. The developed strategy is readily appliable to the construction of chassis cells of other microorganisms.

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Protoplast-Based Transformation and CRISPR/Cas9 Mediated Genome Editing in *Aureobasidium pullulans* and *Hortaea werneckii*

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Fungi of the genera *Aureobasidium* and *Hortaea* are remarkable organisms known for their ubiquitous occurrence and ability not only to survive, but to thrive in extreme environments, that are characterized by various environmental stressors including extremely high and low temperatures, pH values, desiccation, and so on.[1,2,3] These black yeasts exhibit unique morphological and molecular features that contribute to their adaptability and survival in harsh conditions.[3] The particular fungi have gained industrial importance as they are utilized in the production of numerous valuable compounds.

In our study we describe the development of a CRISPR/Cas9 genome editing method for *Aureobasidium pullulans* (EXF-150, EXF-3645) and *Hortaea werneckii* (EXF-15, EXF-562), using a plasmid-based transformation approach in the protoplasts of the mentioned fungi. The targeted integration of DNA cassette carrying resistance to the antimycotic geneticin (*genR*, G418) and the nucleotide sequence for nuclear/cytosolic green or red fluorescent protein at the site of Cas9-induced DSB in genes *ura3*, *ade2*, and *leu2* resulted in auxotrophic fungal strains with antimycotic resistance and the ability to express fluorescent proteins. To screen the presumably transformed strains, we cultivated them on a medium without additional amino acids and employed a PCR-based screening method, to confirm the insertion of the DNA cassette at the correct location in the genome. This method of genetic manipulation could lead to innovative solutions in the fields of medicine, agriculture, and biotechnology in later stages of research. CRISPR/Cas9 technology combined with extremotolerant fungi could also prove useful for studying the limits of life on Earth, and at the same time, potential discoveries could also be applied to extraterrestrial environments.

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Investigating the Molecular Basis of Caesium resistance in *Bacillus* sp. ZR-6 Through Mutagenesis and Genome Sequencing

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Caesium (Cs), an alkali metal with isotopes such as ^{134}Cs and ^{137}Cs, significantly contributes to radioactive contamination [1]. Previous research on *Microbacterium* sp. TS-1, a strain resistant to high concentrations of caesium, revealed two Cs^+ resistance mechanisms [2,3]. In TS-1, the Cs^+/H^+ antiporter CshA expels Cs^+ to maintain intracellular levels below 200 mM. Below this threshold, incoming Cs^+ competes with Mg^{2+}, which stabilizes ribosomes, leading to their destabilization. Therefore, maintaining high intracellular Mg^{2+} levels enhance the stability of the ribosome complex. On the other hand, the Cs^+ resistance mechanisms of other Cs^+-resistant bacteria remain unexplored. Recently, *Bacillus* sp. ZR-6, a novel bacterium resistant to caesium (Cs^+), was isolated from the intestines of American crayfish. This study aims to identify the genes contributing to the Cs^+ resistance of ZR-6 and to clarify their roles, thus advancing the understanding of Cs^+ resistance in bacteria. Initially, chemical mutagenesis using Ethyl methanesulfonate identified a Cs^+-sensitive strain, ZR-6S, via the replica plating method. Subsequently, a revertant mutant, ZR-6R, emerged through spontaneous mutation. Chromosomal DNA from each strain was extracted, and whole-genome sequencing was performed using a next-generation sequencer. Analysis of the genome sequence identified gene mutations and selected genes potentially involved in Cs^+ resistance. The results indicated a 1680 base pair mutation in ZR-6 compared to ZR-6S, and a revertant mutation in the promoter region of a gene encoding a zinc-transporting ATPase (ZosA) in ZR-6R. This mutation likely contributes to the decreased Cs^+ resistance in ZR-6S and suggests its involvement in the Cs^+ resistance mechanism of ZR-6. ZosA, a type of P-type ATPase which includes a diverse group of cation transporters found across all life forms [4], may play a role in Cs^+ transport and efflux in ZR-6.

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Evidence in support of a novel nucleotide excision repair pathway in the *Euryarchaeota*

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DNA is consistently damaged, demanding persistent repair orchestrated by an intricate network of redundant DNA repair mechanisms conserved across Domains. Many archaeal species thrive in extreme environments that render their DNA more susceptible to sustained damage. Paradoxically, most Archaea maintain basal mutation rates akin to that of Eukarya and Bacteria, intriguingly without the discovery of novel DNA repair pathways exclusive to Archaea[1, 2]. The nucleotide excision repair (NER) pathway has been comprehensively described in Eukarya and Bacteria. Given the contribution of NER to overall fitness in other Domains, it is perhaps surprising that no NER-like pathways have yet been identified in the preponderance of Archaea[3]. Therefore, it remains enigmatic as to how most archaeal species preserve genomic integrity in the face of bulky DNA damage. We have identified a set of helicases, endonucleases, and exonucleases conserved across the Euryarchaeota kingdom, which are predicted to comprise a transcription-coupled nucleotide excision repair-like pathway for repair of bulky lesions. Deletion of these encoding genes from the genome of the hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tko*) compromises the cell's ability to withstand bulky lesion-inducing genotoxins and the repair of such. Herein, we investigate the genetic and biochemical role of this NER-like DNA repair pathway in *Tko* that contributes to the repair of bulky damage in the Euryarchaeota.

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Bioprospecting of Polyhydroxyalkanoates-producing Bacteria from Marine Environment

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Polyhydroxyalkanoates (PHAs) are a group of biopolymers with various applications and excellent biodegradability, but the high cost of production has prevented their use. To reduce this cost, there is a prospect for strains with a high PHA production and the ability to grow in low-cost medium as well as easy cell lysis to PHA recovery. In this context, this work is aimed to conducting a bioprospection of bacteria isolated from sea water in South Korea for the potential production of PHA. A diverse group of bacteria were isolated and screened by Sudan Black B and Nile Red A staining. A total of 27 potent PHA-producing bacteria belonging to the genera *Halomonas* spp., and *Cobetia* spp. were characterized. PHA granules in the cells were visualized using a confocal laser scanning microscope. The presence of a PHA synthase gene (*phaC*) was confirmed by PCR. Gas chromatography–mass spectrometry (GC–MS) analysis was performed for confirmation of the PHA fractions with extracted polymers were identified as polyhydroxybutyrate (PHB). Preliminary experiments revealed that the selected marine bacteria have a high salinity tolerance and PHA accumulation activity. The results of this study suggest a high potential for the production of PHAs by bacteria isolated from marine environment.

A Novel Thermophilic Polyhydroxyalkaonate (PHA) Producing Bacterium, *Thermohahella caldifontis* gen. nov. sp. nov., Isolated from Hot Spring

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Microbial polyesters known as polyhydroxyalkanoates (PHA), produced by various prokaryotes, serve as renewable and biodegradable alternatives to petrochemical polymers in many applications, and leveraging extremophiles for PHA production offers distinct advantages. We isolated a novel thermophilic Gram-stain-negative, motile, and rod-shaped strain capable of PHA biosynthesis, designated as SMD15-11^T, from a hot spring in Incheon, South Korea. The strain grew at concentrations of 0.5–7% (w/v) NaCl (optimum at 3%), at pH 5.5–8.5 (optimum at 7.0-7.5), and in a temperature range of 30–60 °C (optimum at 50 °C). Strain SMD15-11^T shared the highest 16S rRNA gene sequence percentage with *Hahella chejuensis* KCTC 2396^T (92.46%). The genome relatedness indices between strain SMD15-11^T and other type species of order *Oceanospirillales* were in the ranges of 64.7-69.2% for ANI, 56.4-62.7% for AAI, and 19.4-43.7% for dDDH, which were significantly below the cut-off values for the species delineation, indicating that strain SMD15-11^T could be considered to represent a novel genus within the order *Oceanospirillales*. The genome comprised 3,444,746 bp with G+C content of 60.1%. Genes involved in PHA synthesis pathway were identified within the genome of the strain SMD15-11^T. The phenotypic, chemotaxonomic, phylogenetic, genomic, and physiological properties indicate that this thermophilic PHA-accumulating strain SMD15-11^T represents a novel genus in the order *Oceanospirillales* for which the name *Thermohahella caldifontis* gen. nov., sp. nov. (=KCTC 8289^T=NBRC 116488^T) is proposed.

Functional Characterization of 4-α-Glucanotransferase and α Amylolytic Enzyme from Hyperthermophilic *Fervidobacterium islandicum* AW-1

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The production of dietary fiber is increasingly becoming a subject of interest in the health food industry. Isomaltodextrin (IMD) is a novel dietary fiber: a type of α-glucan produced from starch using enzymes derived from microorganisms. The combination of starch-active enzymes in the glucanohydrolase and glucanotransferase families has been widely studied to identify enzymes that successfully combined to produce novel functional glucan structures. Here we report the characterization of two thermozymes that could be employed in an effort to develop an efficient process for the production of IMD. α-amylase (FIAmyA) and 4-α-glucanotransferase (FIGTase) from hyperthermophilic *Fervidobacterium islandicum* AW-1 were overexpressed in *E. coli* BL21 using the pET system. The recombinant FIGTase and FIAmyA enzymes exhibited maximal activity at pH 6.0 and 80°C and 65°C, respectively. FIAmya is a Ca-independent α-amylase that has hydrolysis activity against a wide range of substrates including starch, maltodextrin, pullulan, and cyclodextrins. FIGTase converts shorter maltooligosaccharides (MO) to longer MOs but also able to use isomaltose and cellobiose as acceptors. FIGTase with liquefying and transferring activities could be introduced in an effort to improve IMD productivity.

Unveiling Thermophilic Bacteria: Genomic Insights and Medical Applications

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Next generation sequencing has emerged as a powerful tool, both for the biodiversity analysis of complex samples as well as studying metabolic pathways[1]. Microbes constitute a major part of earth's biological diversity and act as a key player by playing an important role in our day-to-day life[2] especially thermophilic microorganisms prevalent in hot environments, These bacteria play a crucial role in various fields, including human health and research, by supplying DNA polymerases for PCR particularly in detection and prevention of various diseases[3], along with other intriguing enzymes. This research project focuses on taxonomic classification of a thermophilic strain through whole-genome sequencing to pinpoint genes encoding enzymes with potential medical applications.

Genomic DNA was extracted from the bacterial culture using a genomic DNA extraction kit. Subsequently, the genome was fragmented, and the fragmented products were amplified to create libraries. Quality control of the libraries was conducted using the Qubit™ 3 Fluorometer and 2100 Bioanalyzer. Sequencing was performed at The ESSBO Genomics Platform on the Illumina MiSeq instrument after loading the samples into the sequencing cartridge. The strain identified through whole-genome sequencing (WGS) possesses a circular genome size of approximately 4,246,735 base pairs (bp) without plasmids, with a GC content of 45.87%. Phylogenetic analysis categorizes this strain into the genus *Bacillus*, specifically identified as *Bacillus licheniformis*, with a bootstrap value of 100 supporting this identification. Whole-genome sequencing emerges as the swiftest and most cost-effective platform for obtaining a comprehensive understanding of a strain's genetic makeup. This information holds significant value across various domains, particularly in the medical field, for exploring and discovering genes involved in diagnostic applications. One potential application is the production of thermostable enzymes for medical research.

Keywords: Thermophilic bacteria, genomic diversity, next-generation sequencing, medical applications, enzyme discovery.

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Molecular acclimation of an alkalitolerant and halophilic deep-sea isolate to icy moon-relevant ammonia stress

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Ammonia-water is present in the subsurface oceans of icy satellites Enceladus[1,2], Titan[3,4], Europa, Ganymede and Calisto[5] which have been long postulated as habitable environments. The limits of life in ammonia has been established for some prokaryotes[6,7], but the specific adaptations have not been well characterised. In this study, we present untargeted metabolomics analysis of halophilic and alkalitolerant deep-sea isolate *Halomonas meridiana* Slthf1 following growth in 0.5% ammonia brine (pH 10), a pH-matched sodium hydroxide brine and nutrient growth medium. By comparison of the ammonia-exposed metabolome with the pH-match and nutrient growth metabolic data, thirteen ammonia-specific changes are evident in *H. meridiana*. Amongst these metabolites are common fatty acids palmitic acid, phosphatidylcholine and phosphatidylserine that alter the structure and function of the membrane. Upregulation of aminotransferase inhibitor D-allo-Isoleucine, and changes to the synthesis of amino acids alanine and aspartic acid, are also evident indicating alterations to protein metabolism and possibly gluconeogenesis. Our data suggest increased production of amino-containing aromatic residues bearing similarity to fluoroquinolone antibiotics that could account for toxicity effects observed from ammonia exposure. In addition to ammonia-induced changes, we also report alkaline adaptations by comparison of metabolic data from ammonia and pH-matched growth against growth in nutrient medium. The results indicate molecular changes that may be important to adaptation and survival in ammonia and alkaline environments applicable to extreme conditions within icy moon oceans and terrestrial habitats.

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Spatiotemporally Stable Microbial Communities Respond to Seasonally Changing Conditions in a Cool-temperate Deciduous Forest Soil

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Soil microorganisms play an important role in the flux of greenhouse gases, such as CO_2 and CH_4, in forest ecosystems. However, the spatial and temporal variations of their metabolic activities remain largely unknown. Here, a single-year seasonal change of the soil microbial community structure and activities in the small ridge and valley sites of a cool-temperate deciduous forest were investigated using atmospheric measurement and high-throughput sequencing of the 16S and 18S rRNA genes and transcripts. Soil-air concentrations of CO_2 were high in summer and at comparable levels in both sites. The CH_4 concentrations at a shallow layer (soil depth: 10 cm) in the ridge site were lower than those in the valley site, while they kept rather stable throughout seasons. Chamber experiment indicated that CO_2 emission and CH_4 absorption were activated in summer. Microbial diversity analyses showed that prokaryotic communities at a surface layer (depth: 0-5 cm) were different from those at a deeper layer (depth: 30-35 cm), while the differences among sites and seasons were not obvious. Eukaryotic communities exhibited large variation only at the surface layers. Comparison of the gene and transcript datasets indicated the metabolic activation of specific microorganisms. Phylogenetic assay revealed that aerobic methanotrophs, especially the family Methylocystaceae, were abundant and metabolically active at the ridge surface soil, relative to the other soils. The results of this study demonstrated that the spatiotemporally stable microbial communities were involved in the CO_2 and CH_4 fluxes under seasonally changing conditions of the deciduous forest soil.

Key words: Soil microorganism, cool-temperate deciduous forest, atmospheric measurement, high-throughput sequencing

The canonical single-stranded dna binding protein ssb is not an essential replication protein but an rna chaperon in *Saccharolobus islandicus*

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Single-stranded DNA binding proteins (SSBs) have been regarded as indispensable factors in all three domains of life since they play vital roles in DNA replication. Herein, we report that genes coding for the canonical SSB (SisSSB) and the non-canonical SSB (SisDBP) in the hyperthermophilic archaeon *Saccharolobus islandicus* REY15A can both be deleted. The cell cycle progression and genome stability of the deletion strains is not impaired, suggesting that SisSSB and SisDBP are not essential for cell viability. We found that Δ*Sisssb* exhibited higher ratio of cell aggregation after NQO treatment indicating that SSB was involved in DNA repair or DNA damage response. Interestingly, at a lower temperature (55°C), the protein level of SisSSB increases ~1.8 fold in the wild type and the growth of Δ*Sisssb* and Δ*Sisssb*Δ*Sisdbp* is retarded. SisSSB exhibits melting activity on dsRNA and DNA/RNA hybrid *in vitro* and unwinding RNA hairpin in *Escherichia coli*. Furthermore, the core SisSSB domain is able to complement the absence of the cold shock proteins CspABGE in *E. coli*, suggesting that SisSSB functions as RNA chaperon. We show that a two-fold overexpression of SisSSB is beneficial to the cell growth at lower temperature. Importantly, these *in vitro* and *in vivo* activities are conserved in SSB subtype SSB-1 in Crenarchaeota species that lack bacterial Csp homologs. Overall, we have clarified the function of the archaeal canonical single-stranded DNA binding protein SSB which does not function as a DNA processing factor, but plays a role in processes requiring dsRNA or DNA/RNA hybrid unwinding. Other potential SSB(s) that might work in the absence of SisSSB is now under investigation.

Analysis of the hyper-vesiculating mechanism of *Shewanella vesiculosa* hm13 Using a curvature-sensing peptide

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Extracellular membrane vesicles (MVs) are lipid nanoparticles produced by almost all bacteria, and their physiology and applications have been attracting significant attention. However, the molecular mechanism of MV production has not yet been fully elucidated. *Shewanella vesiculosa* HM13, a cold-adapted Gram-negative bacterium, produces larger amounts of MVs with less variation in particle size than the related *Shewanella* species and *Escherichia coli*[1]. Therefore, genetic approaches to clarify the molecular basis behind the MV production of this strain are expected to be a clue for elucidating important mechanisms of bacterial MV production and morphogenesis.
Herein, we conducted high-throughput screening of the genes related to MV production by strain HM13 using random transposon mutagenesis and a curvature sensing peptide, nFAAV5-NBD, which can sense a curvature of a lipid bilayer and selectively bind to MVs in the presence of the cells[2]. Screening of 10,100 random mutants resulted in the identification of 16 and 7 genome regions whose disruption by transposon insertion caused hyper- and hypo-vesiculation, respectively. The mutations of the genes identified from hypo-vesiculating mutants decreased MV production by 41% to 79%. On the other hand, disruption of the genes coding for dipeptidyl carboxypeptidase, presumably involved in the quality control of membrane proteins, and small chain of Glu synthase, which produces L-Glu and supplies it for the synthesis of D-Glu, a component of peptidoglycan, caused 2.3- and 2.8-fold increase in MV production, respectively, suggesting that membrane stress due to protein misfolding and loss of peptidoglycan components caused hyper-vesiculation in these mutants. We further characterized these mutants and found that some genes contribute to the formation of the nucleic acid-containing MVs. This study introduces a novel *in situ* screening method that does not require MV isolation and provides insights into bacterial MV formation.

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Elucidation of the Involvement of *Deinococcus radiodurans* DR0042 protein in DNA repair

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Deinococcus radiodurans, a radioresistant bacterium, has an extraordinarily effective DNA repair mechanism[1]. DdrA protects single-stranded DNA termini from nucleases and is believed to be involved in single-strand annealing repair[2]. *D. radiodurans* possesses a paralog of DdrA, called DdrAP (*dr0041*), the function of which remains unclear. The *dr0042* gene is located downstream of *dr0041* and forms an operon.

A *dr0042* deletion mutant showed significantly higher sensitivity to mutagens, such as bleomycin, UV-C, and mitomycin C, than the wild strain. Our objective was to investigate the interaction of DR0042 with DNA and other proteins in vitro and to elucidate its involvement in DNA repair mechanisms. We previously expressed the *dr0042* gene in *Escherichia coli* and found that the native, N-terminal His-tag, and C-terminal His-tag proteins were expressed in large amounts in the insoluble fraction. In this study, we solubilized and purified DR0042 protein using a maltose binding protein (MBP) tag [3]. Adding glucose to LB broth was very effective in increasing protein expression in *E. coli* recombinants. The addition of DTT to the buffers was effective for amylose column purification, with a high yield.

Our study will demonstrate whether DR0042 plays a role in DNA repair by interacting with DNA. We also plan to generate antibodies and analyze the intracellular variation and localization of *dr0042* to confirm changes in the amount and localization of the protein before and after mutagen treatment. Additionally, we will examine the interaction between DR0042 and its associated proteins, such as DdrAP and DdrA, to elucidate the DNA repair process involving DR0042.

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Adaptive response of the holdase chaperones of *Acidithiobacillus ferrooxidans* atcc 23270 to different environmental stresses and energy sources

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Acidithiobacillus ferrooxidans belonging to microbial communities involved in the bioleaching of sulfide ores[1]. In natural and industrial environments, this bacterium must tolerate extreme conditions that can damage the cell proteins[2,3]. This microorganism possesses redundancy of genes encoding holdase chaperones[3]. In this work, we evaluated the transcriptional response of these holdase genes under different conditions involving short and long-term stresses by changes in temperature (30° to 37°C), pH (1.6 to 1.2 or 2.0), oxidative status (1 mM H_2O_2), and under different energic growth condition (iron, sulphur, pyrite, spharelite and chalcopyrite). Cells grown under thermal and oxidative stress conditions showed a generalized upregulation of holdase genes, while short-term stress led less increase in transcript levels with *hsp20.2* and *hsp31* genes showing higher mRNA levels. The gene for Hp31 also showed significant upregulation under acidic stresses and growing with sulfur and sulfide minerals. The *hsp20* variants showed different mRNA levels under different conditions, with *hsp20.2* likely being a global stress determinant. Cells cultured on chalcopyrite showed a similar response to that induced by long- term oxidative stress to peroxide suggesting that this substrate induces oxidative stress. With exception pH 1.2 stress, the stresses led to a significant increase of intracellular ROS content. The analysis of ATP indicated that intracellular concentration does not change in cells under thermal stress, under long-term stress to peroxide and pH 1.2. Since holdase chaperones do not use ATP, the upregulation of this holdase genes under different stress and energy sources suggest that they can constitute a highly flexible network that contribute the correct (re)folding or protection of proteins guaranteeing that ATP is available for other cellular processes. These results help to understanding the proteostasis systems in extreme acidophilic bacteria.

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Adaptive changes in *Fervidobacterium pennivorans* T, a case of in vitro evolution

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The thermophilic bacterium *Fervidobacterium pennivorans* T grows at 70 °C and neutral pH. This strain belongs to the phylum Thermotogota, it is a strictly anaerobic rod, occurring singly, in pairs or short chains and with a calculated generation time of 150 minutes. It is heterotrophic, growing on a variety of sugars and proteinaceous substrates, like glucose or peptone. It is also capable to grow on chicken feathers, breaking down the keratin anaerobically at high temperatures, an unusual feature shared with other members of genus *Fervidobacterium* [1]. It was first sampled and isolated in 2016 in a hot spring of Tajikistan. After multiple culture transfers and selection processes its keratinolytic capabilities enhanced over time, decreasing the degradation time from one week to 48 hours. The variant calling comparison between the original and adapted strains identified a total of 49 genetic differences, 11 of which are overexpressed when growing this strain with chicken feathers. Among these differences we found 14 SNP, 30 frameshift mutations and 8 missense variants, including several proteins which function remain unknown and an amino acid dehydrogenase, also overexpressed during keratin growth. This enzyme may be relevant for the keratin degradation process. This work shows a genomic comparison between the original and adapted strains and the phenotypical and genetic evolution of this bacterium.

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Metagenomic analysis of acid diatomaceous soils from the soos national nature reserve, czechia

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The Soos National Nature Reserve, situated in Western Bohemia, Czech Republic, hosts a wide variety of habitats, including peat bogs, mineral fens, mofettes, and diatomaceous soils, within a relatively small area (211 ha). However, the natural evolution of these sites has been influenced by former human activities, such as mining. The mining of the diatomaceous soils resulted in their high acidification, creating an extreme environment. With a pH between 2 and 4, a high silica and iron content, and a lack of vegetation, diatomaceous soils in the Soos area represent a unique and challenging habitat for microorganisms to thrive[1, 2]. The objective of this study is to analyse the microbial diversity of the Soos diatomaceous soil sites, to understand the metabolic processes within the communities, and to identify the genes associated with the microbial response to low pH.
Two different approaches were used to achieve our objectives – shotgun sequencing of the environmental DNA (metagenomics), and targeted sequencing of 16S rRNA genes and ITS regions (metataxonomics) along with PLFA analyses. The metataxonomic data were used to analyse the composition of the microbial communities, and the metagenomic data provided information on the ecology of the soils and the metabolic potential of the communities therein.
In all four soils studied, bacteria and archaea were the dominant organisms, while eukaryotes represented a minority of the microbial communities. The most abundant taxa belonged to the phyla *Actinomycetota*, *Thermoplasmatota,* and *Pseudomonadota*. Functional analysis is still ongoing, and focuses mainly on autotrophy, iron and silica metabolism, and genes associated with low pH preferences.
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System-Wide Proteomic Analysis of Membrane-Associated Keratin Degrading Protein Complexes in *Fervidobacterium islandicum* AW-1

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The biodegradation of recalcitrant keratin by thermophilic microorganisms emerged as a sustainable and eco-friendly approach to waste management and bioconversion. *Fervidobacterium islandicum* AW-1, an extremely thermophilic, anaerobic bacterium, has been found to degrade native chicken feathers within 48 hours at 70°C through a unique mechanism that involves direct cellular adhesion to the substrate. In this study, we conducted a global proteomic analysis of *F. islandicum* AW-1 under various nutrient conditions and feather-degrading activity to investigate the metabolic pathways and functional proteins involved in keratin degradation. Our analysis identified putative keratinolytic enzymes and revealed significant changes in the subcellular proteome during feather degradation. Additionally, we performed spatiotemporal analysis using activity-based protein profiling with LC-MS/MS, which unveiled the composition and interactions of membrane-associated keratin-degrading protein complexes within the biological system. Our findings provide insights into the intricate and dynamic molecular mechanisms involved in keratin degradation by thermophilic microorganisms and could contribute to the discovery of novel enzymes for various industrial applications.

Identification of a transcriptional regulator, AsnR, involved in L-Asn metabolism in *Thermococcus kodakarensis*

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A putative Lrp/AsnC-type transcriptional regulator, AsnR, in the hyperthermophilic archaeon *Thermococcus kodakarensis* was identified. Disruption of AsnR-encoding gene (TK2110) resulted in increases in transcript levels of multiple genes including those encoding L-asparaginase (*ash*) and ADP-forming succinyl-CoA synthetase (*scs*). Electrophoretic mobility shift assays (EMSA) indicated that the AsnR binds to the *ash*, *scs* and *asnR* promoters. Two binding sites were identified in the *ash* promoter; one 37-bp upstream (motif 1) and another 4-bp upstream (motif 2) of the putative TATA box sequence and overlapping the BRE. Dissociation constants of AsnR with motif 1 and motif 2 were 10 nM and 75 nM, respectively. When concentrations of AsnR were increased, multiple, higher molecular weight complexes were observed with EMSA using the *ash* promoter as a probe. With increasing concentrations of L-Asn, these multiple complexes converged to a single high molecular weight complex. Gel-filtration chromatography suggested that AsnR forms a dimer in solution, but assembles into an octamer with increasing concentrations of L-Asn. *In vitro* transcription revealed that AsnR represses transcription of *ash*, and that addition of L-Asn relieves the repression. Expression of asparaginase in *T. kodakarensis* cells increased in response to increasing concentrations of L-Asn, and this response was dependent on *asnR*. A model is proposed in which AsnR represses transcriptional initiation of *ash* by binding to motifs 1 and 2 as dimers under low L-Asn concentrations. Increases in L-Asn concentration trigger octamerization of AsnR and its release from motif 2, resulting in derepression of *ash* transcription.

Enhancing Ammonia Oxidation in Copper-Contaminated Soils with Methanotrophs

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Copper is a key cofactor in the enzymatic pathways of most microorganisms; however, its bioavailability is extremely limited in the environment, and high concentrations are toxic. This study investigated the effects of copper dosing on ammonia-oxidizing microorganisms (AOM) and methane-oxidizing bacteria (MOB) cultivated at various copper concentrations. The activity of ammonia-oxidizing bacteria (AOB) was inhibited by copper concentrations above 1000 μM, while ammonia-oxidizing archaea (AOA) were inhibited at concentrations as low as 8 μM. However, this inhibition was mitigated through co-culture with MOB. In microcosm experiments using mandarin field soil and wetland samples with copper concentrations high enough to inhibit AOM, methane injection was found to restore ammonia oxidation activity and increase the actual abundance of AOM. This effect was presumably due to the enhancement of methanotrophs by methane injection, leading to the production of methanobactin, which is a copper chelator produced by MOB. The principal component analysis confirmed that the existing high copper content in mandarin fields exacerbated the toxicity of the added copper, lowering ammonia oxidation activity and causing population changes.

These results demonstrate that methanotrophs support the activity of AOM under diverse environmental conditions, including copper-contaminated systems. Overall, this study provides a deeper understanding of the regulation of nitrification through copper and methanotrophs, informing practical applications such as improving nitrification responses in ecosystems and recovering wastewater treatment plants with reduced nitrogen removal efficiency.

Cultivation and isolation of streamer-generating *Ferrovum* spp. populations from acid mine-waters

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Ferrovum myxofaciens, an acidophilic and psychrotolerant species, thrives in extreme environments, such as copper mines and sulfur/pyrite mines[1]. As an obligate autotroph, it relies on ferrous iron as its electron donor and oxygen as an electron acceptor. Moreover, *Ferrovum myxofaciens* synthesizes extracellular polymeric substances (EPS) and thrives in biofilms, often culminating in the formation of microbial stalactites[1]. However, to this date, only one strain of this species has been isolated and characterized. Pivotal phylogenetic analysis of biostalactites found in ore mines and caves located in the Czech Republic and Slovakia indicate the prevalence of *Ferrovum* species, up to 99%, in the biostalactites. This suggests a significant presence and potential ecological role of *Ferrovum* species in these specific cave ecosystems. In order to isolate different *Ferrovum* spp. populations from these biostalactites, multiple cultivation methodologies were employed, including techniques mimicking streamer-like growth and overlay method[1]. These approaches were implemented with the integration of diverse variables, such as different pH levels, concentrations of ferrous ions, and cultivation temperatures. The obtained consortia are currently subjected to sub-cultivation in order to isolate the axenic culture of *Ferrovum* spp. Obtained *Ferrovum* spp. cultures will be subsequently characterized by MALDI-TOF MS and their cultivation requirements will be determined.

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Newly isolated *Bacillus amyloliquefaciens* strains as a producer of bioplastic

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Bioplastics, such as polyhydroxyalkanoates (PHA), offer a sustainable alternative to fossil fuel-based plastics. Derived from renewable non-food resources, PHAs are 100% biodegradable and can match the performance of conventional petrochemical plastics.
However, despite their superior performance compared to other bioplastics like polylactic acid (PLA) and starch-based plastics, the market adoption of PHA is limited due to its higher price point. This elevated cost stems from the expensive raw materials needed for cultivating PHA-producing microorganisms and the costly extraction methods. To reduce production costs, low-value feedstocks such as agro-food waste and surplus streams can be utilized as starting materials.
Two strains, A47 and A48, isolated from saline water in Armenia, were identified as Bacillus amyloliquefaciens via 16S rRNA gene sequencing. Staining and fluorescent microscopy confirmed that these strains accumulate PHA.
Optimization of the cultivation media for high PHA accumulation was conducted, along with investigations into the optimal cultivation time and pH levels. The highest PHA production was achieved using glucose and yeast extract as carbon and nitrogen sources, respectively. The optimal conditions were determined to be a cultivation time of 48 hours and a pH of 7.0.
We employed a solvent-based method for PHA extraction, evaluating chloroform, methylene chloride, and 1,2-dichloroethane under various conditions. Following solvent extraction, cellular debris was removed, and the solution was concentrated via rotary evaporation. The polymer was then precipitated by the dropwise addition of ice-cold ethanol. Current studies are focused on identifying the biopolymer and exploring the cultivation of the strains in whey-based media to further reduce production costs.

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Little islanders: microbiomes of the Kuril Islands hot springs

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The Kurils, an archipelago of 56 islands with 39 active volcanoes, are located between the Kamchatka (Russia) and the Hokkaido (Japan). The hallmark feature of the islands is a large number of hot springs, discharging either on terrestrial or intertidal marine zones. Their microbial communities have been studied fragmentarily, mainly by culture-dependent methods [1-3]. We provided a large-scale screening of 34 terrestrial hot springs, located in Kunashir and Iturup Islands, using 16S rRNA gene V4 fragment amplicon sequencing. All springs clustered into three groups by microbial communities: The Semi-Neutral Bacterial (SNB), Acidic Bacterial (AB) and Acidic Archaeal (AA) groups. The SNB group (pH 5.7-8.5, 40-79°C) showed the highest biodiversity and was almost entirely dominated by bacteria. Cyanobacteria of the *Leptolyngbyaceae* family (3.2-19.9% of the total community) with phototrophic *Chloroflexota,* presenting by *Roseiflexus* (1.8-53%)*, Chloroflexus* (1.8-21.1%) and *'Ca.* Chloroploca*'*(2.4-16.3%) genera, consisted of mats in springs with up to 60°C. The higher temperature ones were dominated by *Aquificota* of *Sulfurihydrogenibium* (3.5-72.6%) and *Hydrogenobacter* (1.3-8.3%) genera. The AB group (pH 2.2-3.6, 41-64°C) was inhabited by representatives of acidophilic bacterial taxa, such as *Acidithiobacillus* (20.5-44.5%)*, Hydrogenobaculum* (6.3-66.1%) and *Thiomonas* (1.3-44.4%). The AA group (pH 1.5-2.9, 50-94°C) was dominated by archaea of *Acidianus* (2.1-28%)*, Metallosphaera* (2.1-29.2%)*, Thermoplasma* (4.7-80.1%)*, Caldisphaera* (3.3-35.9%) genera, as well as of uncultivated lineages, like '*Ca.* Marsarchaeales' (1.6-42.5%), '*Ca.* Caldiarchaeum' (1.5-25%), BSLdp215 (1.1-20.4%). The microbial diversity of the Kuril Islands hot springs correlated with T, pH and water chemistry, but not geographical location.

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Physiological roles of surface polysaccharides In the protein cargo loading and vesiculation Of a hyper-vesiculating bacterium

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Shewanella vesiculosa HM13 is a cold-adapted and Gram-negative bacterium isolated from fish intestines as a hyper-vesiculating strain. Field emission scanning electron microscopic (FE-SEM) observation demonstrated that *S. vesiculosa* HM13 secretes MVs from its outer membrane via blebbing and pinching-off pathways. Interestingly, MVs of this strain carry a single major cargo protein, namely P49[1]. This study focuses on the molecular basis of the selective cargo loading of P49.

The P49-coding gene was found in a gene cluster consisting of genes involved in bacterial surface polysaccharide synthesis (*wza*, *wecA,* and *wzx)*, lipid metabolism (*gdpD* and *lptA*), and protein secretion (T2SS subunit-coding genes). Gene-deletion analysis of the P49-gene neighboring genes showed the mis-localization of P49 in the supernatant fraction (*wecA*, *wzx, gdpD,* and *lptaA*) and cells (T2SS-subunit coding genes), suggesting that P49 passes through the outer membrane via the T2SS machinery and is loaded onto the MVs via the interaction with surface polysaccharides. Previously, we demonstrated that this strain produces O-antigen lacking lipopolysaccharide (LOS) [2], and capsular polysaccharide (CPS) consisting of a 5-sugar repeating unit[3]. The deletion of *wzx* resulted in the disappearance of CPS in both the cells and MVs, and complementation experiments restored the CPS production and appropriate MV localization of P49 to MVs. *In vitro* assay also demonstrated that P49 can bind to CPS directly, indicating that P49 is loaded to the MV surface via direct binding with CPS[4]. We also suggest the generation of new surface-engineered vesicles, which are modified through the *in vitro* binding using modified P49 without any modification of membrane components, such as phospholipids and LOS.

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Characterization of Mutations Induced by DNA Damage in *Rubrobacter radiotolerans*

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Rubrobacter radiotolerans is a gram-positive, rod-shaped bacterium belonging to the phylum Actinomycota with an optimum growth temperature of 45°C. It was isolated from the Misasa hot spring in Japan in 1973[1]. The entire genome was sequenced in 2012. It is known to have the highest radioresistance to any of the currently identified bacteria. Therefore, we referred to this as a hyper-radioresistant bacterium. Nevertheless, no genomic engineering system has been established and the molecular mechanisms underlying radioresistance remain unclear. The primary objective of this study was to explore the mechanisms that allow this bacterium to withstand environmental stress. In this study, we analyzed the mutation rates of *R. radiotolerans*. The purpose of this analysis is to evaluate the efficiency and reliability of the DNA repair system.

Examination of the 10% survival rate after bleomycin treatment revealed that the radioresistance of *R. radiotolerans* was approximately eight times higher than that of the most studied radioresistant bacterium, *Deinococcus radiodurans*. This result indicated that *R. radiotolerans* exhibited high resistance to bleomycin-induced DNA double-strand breaks. Next, we determined spontaneous and bleomycin-induced mutation rates. The mutation rate was measured by obtaining rifampicin-resistant mutants using *rpoB* as an indicator[2]. No significant difference was observed between the spontaneous mutation rate and the mutation rate after bleomycin treatment. Previous studies have shown that *D. radiodurans* exhibits an increased mutation rate under DNA damage stress, which can be attributed to a damage-induced DNA repair mechanism[3]. In contrast, *R. radiotolerans* probably lacks a strand break-inducing DNA repair mechanism.

We are currently investigating the increase in the mutation rate induced by DNA damage stress caused by ethyl methanesulfonate and hydrogen peroxide, and our findings will be presented together with this research.

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Isolation and characterization of extremophilic Bacteria, Haloviruses and Protozoa from Algerian Saharan ecosystem

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Hypersaline, chott and sebkha aquatic ecosystems are a prime model of extreme environments sheltering halophilic microflora that develops at the limits of life. Halophilic protozoa and haloviruses, which infect extremely halophilic Archaea, are also an important group in these extreme environments[1, 2, 3]. So, extreme halophilic Bacteria and Archaea dominate the microbial communities found in these environments. These microorganisms have a biotechnological potential represented in the production of biomolecules of industrial interest. The main objective of this study is to isolate by cultural methods extreme halophilic bacteria strains, halophilic protists and haloviruses from water samples coming from four different Algerian sebkha. Physico-chemical analysis of the samples showed a neutral to slightly alkaline pH and high salinity.

A total of 39 bacterial isolates were selected based on their macroscopic morphological properties. Of this total, 12 strains were selected for polyphasic identification combining phenotypic and molecular characterization. Sequencing and analysis of the 16S rRNA genes of the selected strains revealed that the latter are affiliated with 3 different archaeal families and 7 different genera; Halobacteriaceae (*Haloarcula* and *Natribaculum*), Haloferacaceae (*Halorubrum* and *Haloferax*) and Natrialbaceae (*Natrinema*, *Haloterrigena* and *Halostagnicola*).

As for the biotechnological exploitation, bioactive molecules stable under extreme conditions, which have multiple industrial applications have proved positive for most of these strains [4, 5]. A diversity of halophilic protozoa shows cells forms probably belonging to the genera; *Cyclidium*, *Nitzschia*, *Litonotus*, *Condylostoma*, *Euplotes*, *Cohnilembus*, *Uronema*, *Cladotricha*, *Tachysoma*, *Condylosrom*, *Chaenea* and *Raphidocystis*.While the results of the analyzed PCR products obtained show the absence of halo-viral genomes that affect Archaea in the analyzed cultures.

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Genetic engineering and regulation of the phosphatidylcholine biosynthesis in acetic acid bacteria

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Phosphatidylcholine (PC) is a primary component of membrane phospholipids in most eukaryotes and plays a critical role in maintaining their membrane integrity. Furthermore, PC is also physiologically important in certain bacteria. Deletion of PC leads to abnormal membrane formation and impaired cellular functions in such cells. However, the molecular basis linking the PC deficiency to the membrane disturbance and cellular functional impairment remains poorly understood.
Acetic acid bacteria produce PC from phosphatidylethanolamine (PE) using PE *N*-methyltransferase (PmtA). We previously constructed a *pmtA*-deletion mutant (Δ*pmtA*) from *Acetobacter pasteurianus* SKU1108 and demonstrated that PC is crucial for tolerance to several stressors such as organic acids, detergents, and high temperatures. Given that such phenotypes are all suppressed by the PC complementation, we were inspired to develop a new living cell system based on the SKU1108 strain. This system allowed us to regulate PC production and study how PC content variations influence the membrane physicochemical properties and the stress tolerance of this strain.
Here, we introduced the gene encoding PC synthase (Pcs), a protein that condenses CDP-diacylglycerol with external choline, derived from *Pseudomonas aeruginosa*, into the specific location on the Δ*pmtA* genome. The resulting recombinant was named Δ*pmtA*+*pcs*. PCR and gene sequencing verified that the *pcs* sequence was successfully inserted at the genome location where the *pmtA* sequence is in the wild-type genome. In addition, we formulated a chemically defined medium devoid of choline substrates to prevent basal PC production. We modified the composition of the MP medium[1] previously designed for *Methylobacterium extorquens* and found that the parental and Δ*pmtA*+*pcs* cells grew well in the medium with ethanol as the sole carbon source.

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Isolation of bioplastic degrading bacterial strains and characterization of a corresponding enzyme

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Plastic waste is pervasive and poses a significant threat to the environment. Consequently, there is a growing interest in utilizing bioplastics as alternatives to petroleum-based plastics. A critical aspect of this endeavor is the search for bioplastic-degrading microorganisms and the characterization of their corresponding enzymes.

From plastic-polluted environments in Armenia, we isolated several bacterial strains capable of degrading various types of PHA (PHBH, PHBV, PHB). Screening was conducted on agar plates containing different substrates. Based on growth and halo zones around the colonies, eight active strains were isolated and characterized. All pure strains grow optimally at a temperature of 30-37 °C and pH 7.0. Two strains, L7 and L8, can grow in the presence of up to 7% sodium chloride. The genomes of the strains were sequenced using Illumina. Sequences were processed with Cutadapt (to remove adapters and barcodes), assembled into contigs with SPAdes, and annotated with Prokka to predict putative amino acid sequences. According to the sequencing results, three strains were identified as Priestia aryabhattai, one as Staphylococcus warneri, and four as Priestia megaterium. Due to its capability to degrade all three types of tested substrates, Priestia aryabhattai strain L8 was chosen for further studies. After cultivation with 0.2% PHBH for 72 hours, a bioplastic-degrading enzyme with a molecular mass of 65 kDa was partially purified from the cultural supernatant using anion exchange chromatography. Similarity search indicated the highest similarity of the enzyme to a PHB depolymerase gene from Bacillus megaterium (GenBank AB258388.1).

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Arsenic removal as biogenic scorodite ($FeAsO_4•2H_2O$) using the thermo-acidophilic archaeon *Acidianus brierleyi* with waste Fe-sludge

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Acdianus (Ac.) brierleyi, an aerobic thermo-acidophilic archaeon (pH_{opt} 1.5, T_{opt} 70°C), grows either heterotrophically or autotrophically on S and Fe[1]. Applications of this archaeon in the mining sector include coal desulphurisation[2] and bioleaching[3]. Mining-impacted waters, such as acid mine drainage (AMD) and refinery process waters, often contain toxic arsenic (As), and our research group has extensively studied this archaeon as a medium for accelerating the biogenic formation of scorodite ($FeAsO_4•2H_2O$), a stable mineral form ideal for As disposal. *Ac. brierleyi* simultaneously oxidises soluble Fe^{2+} (as an energy source) and As(III) (as a detoxification reaction), resulting in the production of scorodite from synthetic acidic Cu refinery process water and the mechanism of a two-step biogenic scorodite formation process was proposed[4].

In order to explore a more sustainable production method for scorodite formation, this study attempted to use waste Fe-sludge from the passive mine water treatment plant in Japan as an alternative source of Fe. The Fe-sludge consisted of amorphous goethite ($Fe^{III}OOH$), and *Ac. brierleyi* was found to grow readily in the presence of < 1.0% Fe-sludge, oxidising As(III) to As(V). Based on solid analyses such as XRD, FTIR and EXAFS, the use of Fe-sludge instead of soluble Fe^{2+} effectively improved the scorodisation rate as well as the crystalinity of the product. The induction period during scorodisation with soluble Fe^{2+} was effectively eliminated when Fe-sludge was used instead. It is likely that some of the *Ac. brierleyi* cells and As oxyanions were adsorbed onto the Fe-sludge surface to create a reaction compartment with higher ion concentrations to promote the reaction of As and Fe at the solid-liquid interface.

In conclusion, this study has highlighted the combined utility of thermo-acidophilic archaeon with waste material for the oxidative removal of highly toxic As(III) from mining-impacted waters.

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Stabilization of ribosomes for Cs^+ resistance in high-concentration Cs^+ resistant *Escherichia coli* strain ZX-1

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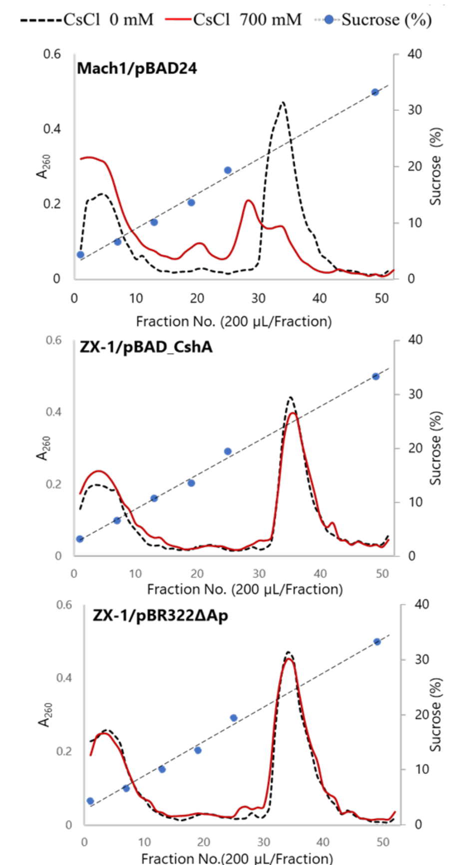
In our laboratory, a high-concentration Cs^+-resistant *E. coli* strain, ZX-1, was obtained, which may enhance the function of the Cs^+/H^+ antiporter (CshA), the major Cs^+ efflux system. Additionally, strain ZX-1 may possess Cs^+ resistance mechanisms other than CshA[1]. Previous reports indicated that excessive Cs^+ can dissociate ribosome complexes[2]. Therefore, the stability of ribosomes was considered crucial for Cs^+ resistance. To evaluate this, the ribosome stability of strain ZX-1 was assessed using sucrose density centrifugation. For the experiments, strain ZX-1 both with and without CshA expression, as well as the Cs^+-sensitive parent strain, Mach1, were used. Each strain was cultured until the optical density at 600 nm reached 0.4. CsCl was then added to one of the cultures to achieve a final concentration of 700 mM, and the cultures were shaken for an additional hour. The cells were lysed at 8000 psi using a French press. The supernatant from the cell lysate was layered atop a sucrose gradient and ultracentrifuged at 36,000 rpm for three hours to separate the ribosomes. As a result, it became clear that strain ZX-1 maintained stable ribosomes under high concentrations of Cs^+, regardless of the presence or absence of CshA. Therefore, the stabilization of ribosomes in strain ZX-1 suggests its involvement in Cs^+ resistance. Whole genome analyses also identified mutations in ribosome-related genes, and future analysis of these genes is planned. This study provides important insights into the mechanisms of Cs^+ resistance and offers a new approach for bioremediation in environments contaminated with Cs^+.



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Ecology and diversity of biological soil crust communities at different elevations in central svalbard (high arctic)

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The High Arctic deserts are extreme polar habitats due to low temperatures, and lack of liquid water and nutrients. However, the various compositions of cyanobacteria, algae, lichens, microfungi and bryophytes create a cover of biological soil crusts (BSCs) which are significant primary producers[1]. This offers a special opportunity to study the mechanisms of their resistance to severe environmental stresses[2].

The ecological and biodiversity research was carried out in 2022–2024. Three sampling plots (Site 1 – Bjørndalen – 47 m a.s.l., Site 2 – Breinosa – 409 m a.s.l., Site 3 – Breinosa – 519 m a.s.l.) were established at different elevations in the vicinity of Longyearbyen in Svalbard. Monitoring of ecological parameters included measurements of air and soil temperature, air humidity, soil water content in upper layers, and chemical analysis of soil (conductivity, pH, water content, and concentrations of NH_4, NO_3, PO_4, Ca, Mg, K, Na, dissolved, and total organic carbon).

Various strains of algae (Chlorophyceae, Klebsormidiophyceae, Xanthophyceae, Zygnematophyceae and Trebouxiophyceae) and cyanobacteria were isolated. The prevalence of cyanobacteria was shown by estimating the biovolume that was also demonstrated in other habitats in Svalbard[3]. Selected strains of algae were chosen for further cryo- and desiccation experiments, cell vitality assessment and transcriptomic studies. The field manipulation experiment included additional water or nutrient supply and winter melt-freeze experiments.

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Anaerobiosis of a Hyperthermophilic Archaeon *Thermococcus onnurineus* NA1

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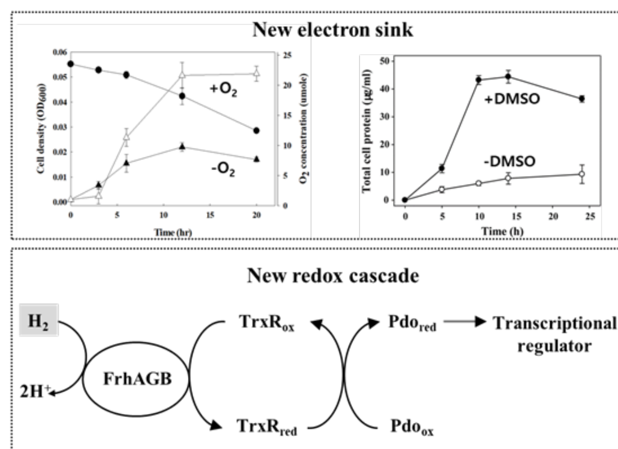
Thermococcus onnurineus NA1 is a hyperthermophilic archaeon that was isolated from sediment collected from a deep-sea hydrothermal vent area. The organism presents a multitude of challenges for cultivation, genetic manipulation, and the study of its oxygen-sensitive proteins due to its anaerobic nature. Despite these obstacles, research into its anaerobic metabolism has yielded fascinating discoveries. Among these findings are the following: (i) Dimethyl sulfoxide (DMSO) can act as an electron acceptor, facilitating the regeneration of the reducing cofactor $NAD(P)^+$, which is coupled with the thioredoxin system.[1] ii) Remarkably, despite its obligate anaerobic status, NA1 is capable of utilizing oxygen (O_2) as an electron acceptor.[2] iii) The overexpression of the *frhAGB* gene cluster has been observed to enhance the O_2 tolerance of NA1.[3] iv) Hydrogen (H_2) can serve as an alternative electron donor for the thioredoxin system, facilitated by the hydrogenase encoded by the *frhAGB* genes.[4] These physiological features of *T. onnurineus* NA1 offer profound insights into the mechanisms of microbial anaerobiosis in extreme environments such as deep-sea hydrothermal vents.



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Genomic Insights into Glacial Microbiomes and Viral on the Tibetan Plateau

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Glaciers are essential for understanding environmental changes, particularly on the vulnerable Tibetan Plateau with its vast low-latitude glacier coverage. Understanding glacial microbiomes and viruses is vital for evaluating ecosystem functions and ecological modeling, especially for the Tibetan Plateau's mountain glaciers, which support approximately 20% of the global population.
From sequencing 85 metagenomes and 883 cultured isolates from 21 Tibetan glaciers, we've developed the Tibetan Glacier Genome and Gene (TG2G) catalog, which represent 968 candidate species spanning 30 phyla. The catalog also contains over 25 million non-redundant protein-encoding genes, the utility of which is demonstrated by the exploration of secondary metabolite biosynthetic potentials, virulence factor identification and global glacier metagenome comparison.
Additionally, we present the Supraglacial Virus Genome (SgVG) catalog, expanding the genomic inventory of 10,840 DNA-virus species from 38 mountain and polar glaciers. These viruses, mainly found in snow, ice, meltwater, and cryoconite, have habitat-specificity and low public health risks. They significantly influence supraglacial microbial communities, with cryoconite hosting the highest viral activity.

The stress reaction of *Fomes fomentarius* to a pulsed electronic field

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Pulsed electric field (PEF) treatment is known to build up a transmembrane potential, which leads to the permeabilization of the membrane of cells. At high field strengths, this leads to inactivation of microorganisms. However, for bacteria treated with a sublethal electrical field, an increase in the maximum specific growth rate and lag phase was observed[1]. The knowledge about the effects of sublethal field strength on fungi is still lacking. In this study, we investigate the influence of PEF on the mycelium of the fungus *Fomes fomentarius.* The composite of the mycelium of this species and solid, wooden particles can be used as a resource-efficient material for industrial applications due to its solid, durable form. Higher growth rates and a higher degree of cross-linking of the mycelium caused by PEF would therefore be beneficial to increase the yield and improve the stability of the material.

Growth behaviour and macroscopic morphological properties were observed exposing the dispersed fungus mycelium to PEF with a field strength between 1 and 3 kV cm^{-1}.

First results indicate that the colony growth increased the most at a field strength of 2 kV cm^{-1}. Macroscopic analysis revealed a higher mycelial density and changes in the colony structure following PEF treatment. The results demonstrate, for the first time, that PEF can impact the fungal growth, which might enhance the material properties of fungal mycelium based composite materials.

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Two new closely related GH13 subfamilies represented by the amylolytic enzymes isolated from extremophiles

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In the CAZy database (www.cazy.org/),[1] the family GH13 is currently divided into 47 subfamilies.[2-4] Such subdivision allows for the highlighting of evolutionary fingerprints, which frequently correspond to subtle functional differences among the diverse GH13 enzyme specificities.[5,6] However, several characterized amylolytic enzymes have still not been assigned to any of the established GH13 subfamilies. This is notably the case of the two characterized enzymes isolated from extremophiles – the maltogenic amylase from hyperthermophilic bacterium *Thermotoga neapolitana*[7,8] and α-amylase from extremely halophilic archaeon *Haloarcula japonica*.[9] The present study addresses a detailed *in silico* analysis of these enzymes, revealing they may represent two novel closely related but distinct groups at the sequence level. Interestingly, both groups share some attributes within the seven conserved sequence regions (CSRs)[5], but on the contrary, other features in CSRs clearly distinguish them from each other. Furthermore, phylogenetic analysis suggests their remote homology to GH13_38 α-glucosidases and structural differences include the length of domain B (a typical GH13 domain protruding out of the catalytic TIM-barrel in the place of the loop 3). The two groups described here are distinct from established subfamilies, hence their characterization and increasing diversity now allows the definition of two novel GH13 subfamilies.

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Lactate fermentation and H_2 production in a *Shewanella strain* from the deep subsurface

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The Iberian Pyrite Belt (IPB) located in the southeast of the Iberian Peninsula, is known for being one of the biggest reservoirs of metallic sulphides in the world[1]. The deep subsurface of the IPB holds an active system where microorganisms promote different biogeochemical cycles that make possible the development of life[2]. The deep subsurface holds many mysteries, one of them is how life thrives and develops under such extreme conditions of pressure, temperature, and lack of nutrients. H_2 is a very good electron donor (-0.42 V) that and used by a wide variety of prokaryotes in deep subsurface environments[3,4]. *Shewanella putrefaciens* T2.3D-1.1 was isolated from borehole samples, 121.8 m deep into the IPBs subsurface and it has been tested positive for H_2 production in anaerobic conditions. Additionally, its genome harbours the necessary genes for H_2 production[5]. In these experiments we have sought to quantify and understand better the H_2 production in this strain in anaerobic conditions. Using lactate as the C and energy source, H_2 detected in the headspace when the electron acceptors (fumarate or NO_3^-) were exhausted. Cultures in the absence of an electron acceptor also yielded H_2. In the presence of fumarate, succinate, acetate, H_2 and CO_2. Without electron acceptor, the detected products are just H_2 and CO_2. The maximum H_2 production detected in the headspace amounted for 0.134 mM. From a thermodynamical standpoint, the fermentation of lactate to H_2 and CO_2 ($\Delta rG'^0$ = 219.3 ± 38.9 Kj/mol) does not allow for high energy yields, unlike the consumption of lactate coupled to the respiration of fumarate ($\Delta rG'^0$ = -103.6 ± 7.6 Kj/mol). Regardless under favourable conditions, like the absence of H_2 in the headspace and the presence of lactate this reaction can turn favourable for *S. putrefaciens* T2.3D-1.1. Despite the low H_2 yields, it is interesting to report that it is possible to see some growth on just lactate, especially in a genus that has been traditionally described as a non-fermenter[6,7]. Given that the lack of electron acceptors could be a plausible scenario in the deep subsurface, the production of small amounts of H_2 could prove very relevant for the microorganism that develop in these extreme environments.

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Metabolism linking arginine and proline in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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The hyperthermophilic archaeon *Thermococcus kodakarensis* displays heterotrophic growth on a variety of organic compounds, including amino acids, as a source of carbon and energy. In catabolic metabolism in *T. kodakarensis*, many amino acids are converted to 2-oxoacids by aminotransferases and/or glutamate dehydrogenase, and then oxidatively decarboxylated by 2-oxoacid:ferredoxin oxidoreductases to acyl-CoAs, and finally used for ATP synthesis by NDP-forming acyl-CoA synthetases. Based on the substrate specificities of these enzymes, the amino acids that are utilized in this catabolic metabolism are clearly defined (Ala, Cys, Glu, Phe, His, Ile, Leu, Met, Gln, Val, Trp, Tyr), whereas the remaining eight amino acids (Asp, Gly, Lys, Asn, Pro, Arg, Ser, Thr) do not appear to be degraded by this pathway[1-5].

We are focused on the interconversion of amino acids in *T. kodakarensis*, particularly those of the latter group that are not subject to the catabolism described above. In this study, we report a new enzyme that is involved in the synthesis/degradation of Pro and Arg in *T. kodakarensis*. We identified an enzyme which we designate "arginine synthetase"[6]. The enzyme converts citrulline, ATP, and free ammonia to Arg, ADP, and phosphate in a reversible manner. Arginine synthetase was necessary in providing ornithine, the precursor for Pro biosynthesis, as well as in generating ATP. The enzyme is widespread in nature, including many bacteria and a few eukaryotes, and catalyzes a long-overlooked energy-conserving reaction in amino acid metabolism.

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Adaptive evolution of *Saccharolobus islandics* REY15A with cellulose as carbon source

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The microbes from *Sulfolobales* could provide potential platforms for lignocellulosic biomass hydrolysis and utilization due to their thermophilic and acidophilic properties [1]. However, these microbes grow poorly on cellulose substrate, and cannot grow on the pretreated lignocellulosic hydrolysate by acid probably due to the low ability to use the substrate or the toxicity of the pretreated hydrolysate. Microbial adaptive laboratory evolution has become a common method for obtaining high-yield and tolerant strains [2]. In order to improve the tolerance of the cells to high concentration of sodium carboxymethyl cellulose (CMC), firstly, we domesticated *Saccharolobus islandics* in CMC containing medium. After about 400 days of continuous domestication culture (253 transfers), a strain designated as E233CD1 was obtained. The tolerance of E233CD1 to CMC increased from 0.4% to 1.1% (w/v). Further, based on the E233CD1 strain, domestication was carried out using pretreated corn straw steam explosion hydrolysate. After about 60 days of continuous culture, we obtained another strain, here named as E233CD-YG1, which can grow on low concentration (0.3%) pretreated corn straw supplementary with 0.1% arabinose. Genome sequencing revealed that a dozen of indels and point mutations occurred in the genome of E233CD1. Genetic analysis (overexpression or knockout of related genes) revealed that one cellulase hydrolyzing enzyme and several transports are partly responsible for the growth improvement of E233CD1. Further experiment of function of five potential genes were carried on.

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The utilization of Alkaliphilic bacteria in weight reduction and degradation of Soy sauce moromi lees and Evaluation of their effects

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Soy sauce lees, a byproduct of soy sauce production, are high in salt and contain 25% fiber, making their reuse costly. This research aims to effectively utilize and reduce the amount of soy sauce moromi lees, which contain highly concentrated salt and are produced annually in Japan at approximately 60,000 tons. In this study, we evaluated the effectiveness of weight reduction in soy sauce moromi lees using alkaliphilic bacteria isolated from soil samples to identify microorganisms suitable for this purpose. Twenty-one soil samples were screened on an alkaline soy sauce lees medium, and four out of one hundred five isolated strains were found to qualitatively decompose dark soy sauce lees. To measure the reduction of dark soy sauce lees in the culture liquid of alkaline dark soy sauce lees, a PTFE membrane filter with a pore size of 10 μm was used. The culture medium, containing powdered dark soy sauce lees, was inoculated with candidate strains and cultured alongside an uninoculated control at 30°C and 200 rpm. Every 24 hours, the culture liquid was extracted, and the weight after filtration was measured. As a result, one of the four candidate strains, No. A5, decomposed ca. 66% of the soy sauce lees in the alkaline whole soybean soy sauce lees medium after five days. Similar experiments were conducted with light soy sauce lees and whole soybean soy sauce lees. About 57% reduction was observed in the whole soybean soy sauce lees. However, less reduction was observed in the light soy sauce lees. When light soy sauce lees were tested in a neutral medium, the decomposition of soy sauce moromi lees was found to be accelerated compared to that in the alkaline medium. Future plans include conducting compositional analyses of soy sauce lees residues and verifying weight changes in soy sauce lees using cultured supernatants.

Bioplastic-degrading mesophiles from plastisphere and thermophiles from the teristerial geothermal springs

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Increasing plastic production and the release of microplastic into the environment underscore the need for circular plastic economy[1]. Microbes, particularly extremophiles, hold great potential for enabling more sustainable technologies through the biodegradation and enzymatic recycling of polymers[2]. In this study, 13 mesophilic and 4 thermophilic strains were analysed from over 80 isolates obtained plastispheres in various plastic polluted aquatic environments and Armenian geothermal springs. The strains were identified based on 16S rRNA genes sequence analyses. Their capability to degrade various bioplastics, such as polylactic acid (PLA), polybutylene succinate (PBS), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV was tested under optimal growth conditions (35°C and 55°C, pH 6.5) using clear zone on agar plates. Strains showing clear zones on PBS and PHBH were identified as representatives of genera *Bacillus, Streptomyces, Parageobacillus* and *Anoxybacillus*. The PLA1-1 strain *Rhizobium pusense* produced a clear zone on PLA plates. The largest clear zones was observed for *Streptomyces griseorubens* Act 2-2 and *Anoxybacillus karvacharensis* K1 strains on PHBH-containing plates (Ø 5cm) followed by *Bacillus subtilis* PLA 2.3.1 and PET 2.2.1 strains on PSB-containing plates (Ø 1cm). Only a few mesophilic strains were able to degrade PHBV. The genomes of the strains *Parageobacillus toebii* H70, *A. karvacharensis* K1 and *Anoxybacillus* sp. H69 were sequenced, reavealing genes encoding esterase/lipase/thioesterase, lipase/acylhydrolase, putative acetyl esterase, carboxylesterase, and some uncharacterized proteases correlated with biodegradation of bioplastics. This work was supported by the ADVANCE Grant provided by the Foundation of Armenian Science and Technology and Yerevan State University.

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Harnessing nature's shield: carotenoids of the antarctic arthrobacter sp. Strain lapm80 for uv-b photoprotection

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Antarctica's adverse conditions, such as high intensities of ultraviolet (UV) radiation, constitute a challenge for microorganisms. To withstand this extreme environment, extremophilic bacteria developed adaptive strategies such as photoprotective carotenoid biosynthesis[1]. Carotenoids with sunscreen properties hold industry significance, given the concern about skin burns and skin cancer caused by UV exposure[2]. Also, there is a demand for sunscreens from natural sources in opposition to synthetic photoprotectors with potential risks for humans and the environment[3]. In this study, we assessed the carotenoid synthesis of isolates from King George Island soils, and the pigment's influence on UV resistance. Carotenoid bioprospection and identification, molecular analysis, and UV resistance were assayed. Among the tested bacterial isolates, one displayed the highest carotenoid content and it was genetically identified as a likely novel *Arthrobacter* sp. strain LAPM80. Synthesis of decaprenoxanthin C50 carotenoid was predicted from the isolate's genome. In addition, the LAPM80 strain exhibited significant resistance to UV-B radiation, and scanning electron microscopy revealed negligible surface changes in the irradiated bacteria, correlating with the higher carotenoid production in the stationary growth phase. Chemical characterization of the LAPM80's carotenoid extract revealed major components as C50 carotenoids. These results highlight the ability of C50 carotenoids of a moss rhizosphere isolate to improve UV-B resistance, potentially useful as natural compounds in the dermo-cosmetic industry.

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Methods and perspectives for discovering enzymes from extreme environments

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Lignocellulosic biomass (LCB) represents a promising renewable resource for the sustainable production of bioenergy and bioproducts[1]. The discovery and application of enzymes able to break LCB recalcitrant structure with improved catalytic efficiency and thermal stability is crucial for the efficient use of this biomass[2]. The main objective of this project is the discovery of new thermophilic enzymes that can be used for the production and characterisation of GHs active on LCB. Here, we combine both culture-independent metagenome sequencing and culture-dependent approaches by performing enrichments on specific biomasses to explore metabolic potential of the microbial communities populating geothermal site in Maronti bay (Ischia, Naples) (T= 60-100°C, pH 8.8). This investigation will provide a complete insight into the phylogenetic diversity and their functional genes involved in breakdown of LCB, as well as scientific basis on the bioprospecting of this metabolic potential for use in sustainable industry.

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Study of the degradation of chloromethane by microorganisms under anaerobic conditions

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Chloromethanes are short chlorinated aliphatic hydrocarbons, widely used as solvents or as intermediates in chemical syntheses (*e.g*. production of silicones) and constitute, after hydrocarbons, the most common pollutants of water and soil. Most chloromethanes are naturally produced by biotic and abiotic processes, but the increase in their concentration is linked to human activity.

Little is known about the microbiological degradation of chloromethane CH_3Cl in the environment, with only a few bacterial strains identified, capable of degrading chloromethane under aerobic conditions, via the *cmu* degradation pathway. Only two additional strains have been found able to degrade CH_3Cl without O_2, *Acetobacterium dehalogenans* strain MC, under acetogenesis conditions, and *Pseudomonas aeruginosa* strain NB1, under nitrate-reducing conditions.

Our project focuses on the study of new microorganisms able to degrade chloromethane under anaerobic conditions, through bioprospecting of sediments from a brackish pond on the French Mediterranean coast. Enrichment cultures were established from the sediments, to target anaerobic microorganisms, in particular sulfate-reducers. These cultures exhibit degrading chloromethane activity. The microbial diversity of these cultures was analysed. This will involve isolating strains of interest from these cultures, which could be used in biotechnological processes. The longer-term aim of this research is to highlight new pathways for the degradation of chlorinated hydrocarbons.

A Global Deep-Sea Database and Roadmap To Drug Discovery

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Marine life, especially extremophiles—organisms thriving in extreme environments—are a key source of novel pharmaceutical compounds. Natural compounds from the deep sea, adapted to extreme conditions, make extremophiles prime candidates for bioactive compounds with pharmaceutical potential.[1,2] Despite advancements in marine natural products (MNPs) chemistry, open-access databases on MNPs are limited. Extremophiles, due to their rarity and challenging habitats, are less explored, making comprehensive data crucial. We established the DEEPEND marine natural products database through thorough manual curation.

The database hosts approximately 3,000 novel and/or bioactive chemical compounds from the deep sea (below 200 meters), characterized by physicochemical, pharmacokinetic attributes, and biological activities. This includes compounds from extremophiles, offering promising new chemical entities. The database provides geographic, depth, taxonomic, and phylogenetic maps, serving as a platform for verifying uniqueness, identifying potential lead compounds, and exploring structure-activity relationships.

Dengue affects around 400 million people annually. While most infections are mild, about 100 million experience severe symptoms, leading to up to 40,000 deaths yearly. Specific antiviral medications for dengue lack approval, and vaccines do not offer uniform protection.[3] To address this, the Virtual Computational Screening method uses the DEEPEND MNP database to discover potential antiviral compounds, aiming to identify novel agents from extremophiles.

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Hydrogen production from industrial wastes using new Hyperthermophilic organisms isolated from shallow Hydrothermal fields of volcanic islands of the aeolian Archipelago, italy

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One of the alternative treatments for organic industrial waste is its degradation by anaerobic digestion, with the resultant output of hydrogen (H2) generation. The goal of this work is to optimize the H2 production by dark fermentation of various organic industrial wastes such as paper mills, fisheries wastes, straw, and cow manure. As part of this project, new anaerobic and thermophilic (> 70°C) H2-producing micro-organisms, able to degrade organic industrial wastes with low pH (pH 6), were sought within the shallow hydrothermal vents (SHV) of Vulcano and Panarea, which can be both acidic and hot (pH 2-5 and up to 140°C, respectively). Samples from diverse sites around these SHVs were used to inoculate synthetic media with 5 g.L-1 of industrial wastes as substrates. Enrichment cultures were incubated at 80°C and pH 6.0 under anaerobic conditions in 50 mL glass bottles to enrich H2-producing hyperthermophiles. Growth and H2 production were monitored daily by microscopic cell counting and gas chromatography. The best results among the industrial substrates tested were obtained with cow manure, reaching up to 5 mM H2. These cultures showing significant H2 production were further tested in a 1 L bioreactor shaken at 500 rpm and operating in fed-batch mode under the same conditions (anoxia, 80°C, and pH 6) in the presence of industrial substrate at 5 g. L-1. Microscopic observations showed stable microbial consortia with two distinguishable morphotypes consisting of irregular cocci and rodshaped cells. The Identification of the consortia members grown in bioreactors and bottles by sequencing their 16S rRNA gene is in progress and will be presented.

Unveiling a novel DNA repair mechanism featuring DdrA

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Deinococcus radiodurans exhibits exceptional resistance to radiation, which can be attributed to its distinctive DNA repair capabilities [1,2]. Aiming to develop techniques for minimizing the harm caused by radiation, numerous studies have been conducted to reveal its DNA repair processes. Although various radiation-induced proteins have been identified from *D. radioduran*, their function in DNA repair processes is not fully understood. One of these proteins, DdrA, is similar to Rad52, a eukaryotic protein that plays a role in DNA double-strand break repair and homologous recombination [3]. Despite the similarities, the specific role of DdrA in DNA repair remains unclear. To better understand the function of DdrA in DNA repair processes, we generated mutant strains of *D. radiodurans* that lacked *ddrA*, *ddrAP* (a paralog of *ddrA*), and *dr0042* (a gene located downstream of *ddrAP* in an operon encoding a metallophosphoesterase), and investigated their effects on DNA damage. The *ddrA* deletion strain exhibited slight sensitivity to UV-C, whereas the *dr0042* deletion strain showed high sensitivity to all DNA-damaging agents tested. Surprisingly, the double-deletion strain of *ddrAP-dr0042* displayed the same level of sensitivity as the wild-type strain. Considering the details and traits of the DdrA protein, we propose the following DNA repair model. DdrA serves as a DNA repair protein that is rendered inactive by DdrAP. DR0042 manages DdrA by influencing its interaction with DdrAP. To investigate the functions of DdrA, DdrAP, and DR0042, they were expressed in *E. coli*. DdrA and DdrAP were expressed in the supernatants and antibodies were produced. DR0042, which forms inclusion bodies, was denatured with guanidine hydrochloride. After purification, antibodies will be raised using it as an antigen. These tools can be used to further investigate the functions of DdrA, DdrAP, and DR0042.

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Closing the gap between environmental and laboratory pH in *Acididesulfobacillus acetoxydans*, a moderate acidophilic sulfate reducing bacterium

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Acid mine drainages (AMD) pose a severe environmental threat due to their low pH and high concentrations of heavy metals. Acidophilic sulfate-reducing bacteria (aSRB) can attenuate AMD characteristics through sulfate reduction to sulfide, a proton-consuming reaction at low pH. The produced sulfide precipitates metals, removing them from the liquid phase. As so, aSRB have gained attention for bioremediation and biomining processes. However, it seems to be a mismatch between environmental and laboratory activity at the lowest pH values. Initial isolation trials obtained neutrophiles or acidotolerant SRB which could not grow at pH lower than 3.8-4.0. This raised the question whether aSRB really existed, with suggestions that they might only thrive in micro niches in biofilms, sediments, etc. but not fully exposed to high proton concentrations. We hypothesized that this discrepancy originates from culture conditions rather than metabolic aSRB limitations. We used *Acididesulfobacillus acetoxydans*, an aSRB isolated from AMD sediments with an overlaying water column of pH 2.3. We operated triplicate pH-controlled CSTRs with a dilution rate of 0.015 h^{-1}. We obtained steady states at pH 5.0 (its optimum pH), 4.4, 3.8, 3.5 and 3.2. Mimicking AMD conditions, the three reactors were exposed at pH 3.2 with 3 ranges of Fe, Ni, and Cr concentrations showing a high correlation for metal exposure and growth. Furthermore, a steady state was reached at pH 2.9 for one CSTR with a lowered dilution rate (0.01 h^{-1}). From this CSTR we obtained a culture which showed activity at pH as low as 2.5 in flask cultivations. *A. acetoxydans* did neither form biofilms or aggregates, growing completely planktonic. Samples for transcriptomics were taken at each steady state to track the gene expression profiles throughout the continuous cultivation. Remarkably, there was an increase in the K^+-transporting ATPase encoding *kdpABC*, mechanism shared with extreme acidophiles. Additionally, membrane permeability might be altered at low pH, as an increased core lipid saturation was observed at lower pH values. Interestingly, the lipid priming precursor shifted from leucine to valine. Hence, aSRB handle low pH by impeding proton influx through adopting a more rigid and energy-efficient membrane, and establishing a chemiosmotic gradient.
In conclusion, this study showed the resistance of *A. acetoxydans* to high proton stress while growing at pH as low as 2.9 and being metabolically active at 2.5. These pH values are in the range of the pH found in AMD conditions showing the metabolic potential of aSRB in biohydrometallurgy.

Serpentinization as the source of energy, electrons, organics, catalysts, nutrients and pH gradients for the origin of LUCA and life

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Serpentinization in hydrothermal vents is central to some autotrophic theories for the origin of life because it generates compartments, reductants, catalysts and gradients. During the process of serpentinization, water circulates through hydrothermal systems in the crust where it oxidizes Fe (II) in ultramafic minerals to generate Fe (III) minerals and H_2. Molecular hydrogen can serve as a source of electrons for the reduction of CO_2 to organic compounds, with suitable catalysts present. Using catalysts during serpentinization H_2 reduces CO_2 to formate, acetate, pyruvate, and methane. These compounds represent the backbone of microbial carbon and energy metabolism in acetogens and methanogens, anaerobic chemolithoautotrophs that use the acetyl-CoA pathway of CO_2 fixation and that inhabit serpentinizing environments today. Serpentinization generates reduced carbon, nitrogen and probably reduced phosphorous compounds that were likely conducive to the origins process. It gives rise to microcompartments and proton gradients to support chemiosmotic ATP synthesis by the rotor-stator ATP synthase. This would help to explain why the principle of chemiosmotic energy harnessing is more conserved than the machinery to generate ion gradients via pumping coupled to exergonic chemical reactions, which in the case of acetogens and methanogens involve H_2-dependent CO_2 reduction. Serpentinizing systems exist in terrestrial and deep ocean environments and were probably even more abundant on the early Earth. Serpentinization once occurred on Mars and is likely still occurring on Saturn's icy moon Enceladus, providing a perspective on serpentinization as a potential source for life on other worlds.[1]

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Crispr goes psychrophilic: development of genome engineering tools for the psychrophilic *Pseudoalteromonas haloplanktis* tac125

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Pseudoalteromonas haloplanktis TAC125 (*Ph*TAC125) stands out as a model organism of Gram-negative psychrophilic bacterium which can optimally grow at 15°C[1]. The high quality *Ph*TAC125 genome annotation and the set-up of a psychrophilic genetic toolbox [2,3] has facilitated studies of its peculiar physiochemical/biochemical features, responsible for its cold lifestyle preference[1]. Furthermore, *Ph*TAC125 possesses several properties which makes it a promising host for the recombinant production of human difficult-to-express proteins (e.g., *h*NGF, *h*CDKL5)[4,5]. Towards the exploitation of *Ph*TAC125 as an unconventional cell-factory, the development of CRISPR-based genome engineering tools tailored for *Ph*TAC125 is of paramount importance. Indeed, the introduction of the Antarctic bacterium in a systematic genome engineering pipeline could allow the evolution of novel strains with improved performance. We sought to develop a CRISPR-based tool harnessing Cas9 nuclease orthologues, namely SpCas9 and Sth1Cas9, active at temperatures ≤ 20°C. The system was set up through the development of plasmids featuring two psychrophilic inducible promoters for the expression of Cas9 and a programmed single-guide RNA. To validate the system, a genome-targeting assay was performed targeting three genes (*PSHAa2060*-lon, *PSHAa2062*-clp, *PSHAa0069*-ptrb) on the genome of *Ph*TAC125. This system showed efficient CRISPR-based targeting in *Ph*TAC125, revealing a significant colony reduction when targeting the genome. Subsequently, both Cas9 nucleases will be used for genome editing through homologous recombination, achieving for the first time CRISPR-based gene deletion in *Ph*TAC125. These findings represent enabling advances in the application of CRISPR-based editing in psychrophiles bacteria.

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PerR functions as a redox-sensing transcription factor regulating metal homeostasis in the thermoacidophilic archaeon *Saccharolobus islandicus* REY15A

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Thermoacidophilic microorganisms thrive in environments with high temperature and low pH where the cells are prone to severe oxidative stress due to elevated reactive oxygen species (ROS). While the oxidative stress responses in bacteria and eukaryotes have been extensively studied, less is known about the mechanism in archaea. Here, we report that SisPerR, the homolog of bacterial PerR in the thermoacidophilic archaeon *Saccharolobus islandicus* REY15A, is responsible for the oxidative stress response and transcriptional regulation against ROS. By a combination of genetic, transcriptomic and ChIP-seq analyses, we show that the expression of Dps, NirD, VIT1/CCC1 and MntH which are predicted to be involved in the regulation of cellular metal ion homeostasis, but not that of ROS scavenging enzymes, is significantly up-regulated with H_2O_2 treatment and *sisperR* deletion. Consistently, the expression of Dps, NirD, VIT1/CCC1 and MntH is repressed in the SisPerR overexpression strain. The genes coding for Dps, NirD and MntH are the direct targets of SisPerR as revealed by ChIP-seq and *in vivo* promoter report assay. The study has established that SisPerR is a repressive redox-sensing transcription factor regulating intracellular metal ion homeostasis in *Sa. islandicus* for the oxidative stress defence, thereby expanding our understanding of microbial adaptation to extreme environmental conditions. The mechanism of SisPerR in ROS sensing and regulating gene expression is under investigation.

What phylogenetic novelty hides the thermal springs of Carlsbad?

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Czech warmest thermal springs are situated in the renowned spa town of Carlsbad, which lies on a fault separating the Saxothuringian and Teplá-Barrandian tectonostratigraphic zones in the Bohemian Massif. This fault, terraced bedrock, and alkaline magmatism led to the development of volcanic activity and subsequent mantle degassing, resulting in the formation of bicarbonate-sulfate-chloride-enriched waters containing gaseous and dissolved CO_2 [1]. These thermally heated waters represent the extreme aquatic environment with low nutrient content and lack of human impact, providing us with a great opportunity to investigate a continental subsurface microbial dark matter that accounts for 30% of all prokaryotes on Earth[2]. Moreover, this type of habitat may resemble an early Earth and hide hitherto undescribed prokaryotes that may be part of deep-branching taxa, thus helping to adjust the position of the last common ancestors in the phylogenetic tree[3]. The aim of this study is to analyze the phylogenetic novelty of the prokaryotes inhabiting the Carlsbad thermal springs and to clarify their ecological role in such an extreme environment.

In this study, we focus on the microbial community composition of four thermal springs that differ in temperature and chemical composition. To study phylogenetic novelty, we used three different approaches. First, we used the modified cultivation techniques to obtain isolates of novel taxa. Second, we used 16S rRNA gene amplicon sequencing to reveal the broader novelty potential in our samples, on the basis of which we used the third approach, metagenomic sequencing.

So far, we have recovered 4 members of novel species through cultivation techniques, whose genomes have been sequenced. Amplicon sequencing also revealed a great potential for phylogenetic novelty in terms of hitherto uncultivated prokaryotes and a high abundance of Archaea, especially in the warmest springs. These particular results led to a metagenomic study that confirmed the presence of novel archaeal orders. The ecological role of these taxa is further characterized.

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Exploring the sediment-associated microbiome of etoliko lagoon

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Etoliko Lagoon, located in Western Greece, is an extreme environment characterized by a lack of oxygen, high sulfate concentrations, and a permanent thermocline separating the water column. Despite these conditions, the lagoon supports a diverse range of microorganisms, including putative new bacterial and archaeal phyla. This study investigates the prokaryotic communities in the lagoon's sediment. Extracting high-quality DNA from the sediment poses a significant challenge due to various inhibitory factors, limiting traditional metagenomic analysis. To tackle this issue, a two-pronged approach was employed.

Firstly, next-generation sequencing (NGS) was used to sequence the V3-V4 region of the 16S rRNA gene from the extracted DNA. The results revealed many unassigned Operational Taxonomic Units (OTUs) suggesting the presence of potentially novel taxa. This finding implies that the lagoon's sediment could be a significant reservoir of undiscovered microorganisms. To further explore the taxonomy, the full length of the 16S rRNA gene was sequenced. Cross-referencing results from both platforms provided a thorough characterization of the microbial communities in the anoxic sediments of Etoliko Lagoon.

Secondly, culture-dependent techniques were used to isolate viable strains from different layers of sediment. Cultivation strategies were implemented to simulate the lagoon's anoxic environment. These included solid culture media incubated in special oxygen-deprived chambers, and liquid cultures grown in tightly sealed bottles where oxygen was substituted with a nitrogen-carbon dioxide gas mixture. Anaerobic species like *Clostridium sulfidigenes*, *Paraclostridium benzoelyticum*, and *Terrisporobacter petrolearius* were cultivated and their genomes were sequenced using the Oxford nanopore platform. These data will help to better understand their physiology and metabolic capabilities.

Liquid cultures, used as a proxy for the lagoon's microbial diversity due to the difficulty of direct metagenomic analysis of the sediment, contained a variety of carbon sources to encourage and enrich distinct microbial populations. DNA extracted from these cultures will undergo metagenomic analysis to provide insights into the lagoon's microbial communities' functional potential.

This research not only illuminates the unique ecosystem of Etoliko Lagoon but also provides broader implications for understanding microbial life in Earth's anoxic environments, some of the largest yet least explored ecosystems and bioprospecting.

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Metabolic characterization of novel and uncharacterized prokaryotic lineages in Jáchymov hot springs

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Microbial communities in extreme environments are a valuable source of unique and potentially novel life forms. These environments often have very limited concentrations of nutrients, selecting for microbes that have adopted very specific metabolic pathways to survive. Here, we investigated the microbial community residing in subsurface water springs found in an old silver and uranium mine in Central Europe - Jáchymov, Czech Republic. Using metagenomics, we were able to reconstruct several almost complete or highly complete genomes of the present prokaryotes. Among the identified microbial genomes, several members of novel or uncharacterized phylogenetic groups were identified. The present work focuses on these MAGs, their precise taxonomic placement, and their further characterization with respect to metabolic capabilities. A common feature of the aforementioned MAGs is the presence of the Wood-Ljungdahl pathway (WLP), an ancient pathway capable of reversible CO2 reduction. This pathway is used in carbon fixation and methanogenesis or, in the opposite direction, in the oxidation of acetyl-CoA, methane, or other short-chain alkanes. Carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS), a key enzyme complex of the WLP, is present in both domains of prokaryotes. However, it can be separated based on the specific subunits involved. In this study, we report the presence of an unusual arrangement of the bacterial CODH/ACS gene cluster. Based on our findings, we hypothesize that these forms of the Wood-Ljungdahl pathway are the result of divergent evolution, possibly through horizontal gene transfer, in spatially isolated ecosystems.

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A Strategic Blueprint for the Domestication of *Geobacillus stearothermophilus* as a Thermophilic Platform Cell using the DNMB Suite

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Geobacillus strains from the Bacillales order are facultative anaerobic thermophiles with significant industrial applications, particularly in producing thermostable enzymes for sugar utilization and degrading complex carbohydrates. Using the DNMB Suite and an expanded genetic toolbox, we developed a plasmid artificial modification-based conjugation system and effectively engineered the genetically challenging *Geobacillus stearothermophilus* with GeoCas9EF. This engineered strain demonstrates optimized metabolic pathways, showing great promise for biotechnological applications. The domestication strategies presented here provide a framework for developing robust microbial platforms and encourage exploring diverse species for industrial uses. Particularly, the utility of *G. stearothermophilus* in high-temperature processes, such as producing D-tagatose and other rare sugars, highlights its potential in food-grade applications and directed evolution. This research advances the field of thermophilic microbiology, highlighting the vast potential of thermophiles for both theoretical and practical applications. The future perspectives outlined suggest exciting opportunities for innovation and further advancements in thermophilic applications.

A novel application for extremophilic enzymes

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Biofilms present a significant issue in modern society. They cause issues in several areas including agriculture, medicine, and industry[1]. Biofilms are prolific in the medical industry where it is estimated that 80% of bacterial infections are biofilm related[2] .Many of the current methods of treatment for biofilms are unsustainable due to their use of harsh chemicals and antibiotics. An enzymatic treatment of biofilms is a promising alternative to the current treatment. A number of studies have demonstrated the efficacy of enzymes for treatment of biofilms, but very few have looked at utilising extremophiles for biofilm treatment. There is a huge amount of genomic data freely available for extremophiles enabling ease of mining. Using a variety of microbiological and biochemical techniques, one hyper thermophilic enzyme mined from a thermophilic database (HotZyme[3]) has been characterised and proven to be an effective treatment for biofilm inhibition, as well as a potential anti-virulence treatment.

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Role of diverse lipid molecules in the membrane physiology of acetic acid bacteria

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The membrane of acetic acid bacteria (AAB) features a notably complex lipid composition, including not only phospholipids (PLs) but also hopanoids (sterol-like compounds), sphingolipids, and amino-lipids. This lipid diversity is postulated to be crucial for enabling AAB to adapt to a variety of stress conditions like those encountered during acetic acid fermentation. Despite their apparent significance, the physiological roles and the mechanisms of action of these lipid molecules remain largely unknown.

We have proposed AAB as an ideal model for investigating cellular membrane functions due to their unique lipid composition. We have explored the responses of various *Acetobacter* mutant strains, each deficient in a specific lipid-related gene, to diverse environmental stresses. Our aim is to elucidate the specific contributions of each lipid molecule to membrane integrity and function under such conditions.

Moreover, AAB synthesize phosphatidylcholine (PC), which constitutes over half of the membrane PLs, presenting a unique research avenue. We have developed an *Acetobacter* PC-deficient mutant (Δ*pmtA*) by deleting the phosphatidylethanolamine *N*-methyltransferase gene. This mutation resulted in significantly impaired growth and increased sensitivity to environmental stressors, including ethanol, acetic acid, salt, low pH, and heat. To address the loss of PC, we introduced PC synthase (Pcs) into the Δ*pmtA* cells, allowing the mutant cells to synthesize PC by condensing CDP-diacylglycerol with external choline. Intriguingly, even under minimal choline conditions, Δ*pmtA* cells expressing Pcs restored their growth to the wild-type level either with or without ethanol and showed acetic acid tolerance comparable to the wild-type level. However, the thermo-sensitivity was not restored under the same conditions. These results demonstrated that even relatively small PC levels can partially complement the phenotypes associated with the PC loss, suggesting that PC plays specific roles in growth and stress tolerance in AAB. Additionally, our findings indicated that PC may interact with membrane proteins involved in acetic acid efflux, modulating their functions.

Identifying Transcriptional Regulatory Networks in the Extreme Thermophile *Thermus thermophilus*

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Like most all organisms, the extreme thermophile *Thermus thermophilus* HB8 responds to environmental changes through changes in gene expression programs. These changes primarily occur through transcriptional regulation, with transcription factors, those proteins that recognize specific elements in gene promoters and affect the general transcription machinery, being the primary players. We have developed a general iterative selection method, Restriction Endonuclease Protection, Selection, and Amplification (REPSA), that allows one to determine the preferred DNA-binding sequences of most any ligand[1]. Using bioinformatics, this information can be used to postulate genes regulated by a transcription factor and potential biological functions. We have previously used REPSA to identify the consensus binding sequences and biological functions of several transcription factors[2-9]. Here, we describe the DNA-binding specificity and the regulatory network for the *T. thermophilus* HB8 DtxR homolog TTHA0754. We found that TTHA0754 binds to the *TTHA1941* promoter and negatively regulates the expression of a potential ZIP-family manganese importer in the presence of high cellular manganese concentrations. Thus, we identify TTHA0754 as *Tt*MntR, a manganese transport regulator in *T. thermophilus*.

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Unraveling the temperature-responsive physiology of *Parageobacillus thermoglucosidasius*

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A promising thermophilic platform organism is *Parageobacillus thermoglucosidasius*, as extensive research has been done for its potential in second-generation bioconversion[1,2]. Although basic and more advanced genetic tools have been developed for this species, there is still the need of a deeper understanding of the physiological response to temperature, which is crucial for industrial applications[3,4,5].

In this research, the relationship between temperature and growth rate is investigated to determine the optimal conditions for growth. Moreover, the viability of *P. thermoglucosidasius* in response to various heat shock conditions is examined through SPOT tests. Through RT-qPCR experiments the expression of heat shock genes is investigated to assess at which temperature a heat shock response is most significant. Finally, microscopy experiments in combination with RT-qPCR experiments are used to elucidate the conditions that trigger sporulation in this species.

These findings deepen our understanding of the physiological response to temperature in *P. thermoglucosidasius* and pave the way for optimizing industrial processes with this organism.

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Stsv induced immune response in *Sulfolobus tengchongensis*

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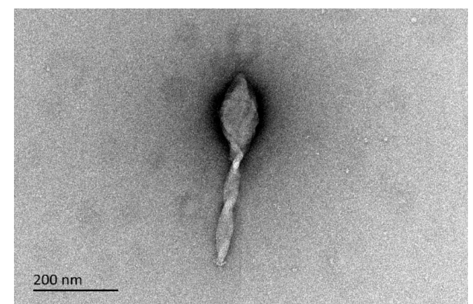
How cells of *Sulfolobus* respond to virus infection and how they establish successful adaptive immune response to clear virus are not fully understood. By using recently established new strain of *Sulfolobus tengchongensis,* RT2, and two new trains of STSV that could infect RT2, we investigated immune response of RT2 upon STSV infection. RT2 has a circular genome of 2744695 bp (accession number: CP146016), and it carries complexed CRISPR-cas genes, including four cas gene cassettes: one Type III-D, one Type I-A, one Type III-B and one Type I-D, and 11 CRISPR arrays, in which 434 spacers are stored. None of RT2's spacers target any strain of STSV. New STSV strain has a spiral spindle-shaped morphology with variable tails in length located in one end and tail fibers mounted at the tail end, which is similar to STSV1 but with distinctive structure features. Upon STSV infection, RT2 paused proliferation, but about five days after infection, RT2 started to regain growth and it eventually cleared virus. New spacer acquisition was detected as early as day 2 after virus infection, but never in day 1. A total of 13 new spacers were captured after STSV infection, and all were stored in type III CRISPR arrays. Eight of the new spacers targeted STSV sequence, however three spacers were found to target itself. A conserved GTN PAM was identified. The culture survived STSV infection with newly captured spacers, and it has acquired adaptive immunity to STSV. Our study revealed that cells of *Sulfolobus* were able to re-organize their cell program in response to virus infection and induced a successful immune response to clear virus. Detailed cell biology studies are undertaking to identify key regulators induced by STSV infection, which will help us to understand how *Sulfolobus* survives in the field.



Micrograph of STSV

The impact of genomic location on ectopic integration and gene expression of a heterologous gene cassette in *Sulfolobus acidocaldarius*

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Sulfolobus acidocaldarius, a thermoacidophilic archaeon, has emerged as a promising candidate for biotechnological applications due to its unique biochemical properties and genetic robustness. Recent advancements in the development of genetic tools and molecular techniques have facilitated the generation of unmarked knock-in/out mutants in *S. acidocaldarius*, relying on homologous recombination and uracil auxotrophy as a selectable marker. The same method can be used for the ectopic integration of heterologous genes into its genome. However, in this case, there is still a lack of understanding if and how the choice of genomic location affects the integration efficiency, as well as the global transcriptional level.
In this study, we selected 11 target locations in the genome of *S. acidocaldarius* SK1 strain, guided by differences in the location with respect to open reading frame orientation (either inter- or intragenic), expression level and essentiality of nearby/overlapping genes and chromosomal structure compartment. Furthermore, we have established an efficient system for genome integration, including the development of a pRN1-based shuttle vector pYX2304, an efficient cloning method for the preparation of the 11 plasmid constructs using the Golden Gate cloning method and optimized transformation conditions in *S. acidocaldarius*. The integration cassette consisted of the *lacS* gene as a reporter, providing simplified selection in both cloning and pop-in/pop-out stages, under the control of a synthetic constitutive *Sulfolobus* promoter. Nine of 11 integration mutant strains were successfully obtained, while two of the strains failed due to either the absence of the target fragment or persistent contamination with the wild-type strain. We assessed pop-in efficiency at each location by counting blue and white colonies and calculating their ratio. Mutant strains were cultivated to early exponential phase followed by gene expression analysis. qRT-PCR results demonstrated significant differences in tha *lacS* transcription level depending on the genomic location, with a remarkable high gene expression level at the *slaA* (*Saci_2355*) location encoding the S-layer structural protein location. We aim to further assess gene expression levels with β-galactosidase assays.

Study of chitin metabolism of the psychrophilic bacterium *Moritella marina* by proteomic analysis

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Chitin metabolism in marine bacteria has not been studied in depth. We used the psychrophilic bacterium *Moritella marina* to determine protein expression changes associated with the presence of chitin[1-4].

M. marina cells were cultured in the presence and absence of chitin, and total cell extracts and secretomes were collected. Proteins were reduced, alkylated and digested with trypsin. The resulting peptides were analysed by liquid chromatography coupled to high resolution mass spectrometer.

Proteomic analysis of total cell extract and secreted proteins revealed numerous proteins involved in chitin degradation and metabolism. The proteomics results were analyzed with bioinformatics tools to place the findings in specific metabolic pathways as well as in the wider context of marine bacterial metabolism.

Our future plans are to validate these preliminary findings with enzymatic and biochemical approaches and perform a metabolomics analysis. Thus, we will obtain a global understanding of chitin metabolism in *M. marina.*

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