EXTREMOPHILES 2022
LOUTRAKI \ GREECE

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HELLENIC REPUBLIC
National and Kapodistrian University of Athens

DEMOKRITOS

13TH INTERNATIONAL CONGRESS ON EXTREMOPHILES

SEPTEMBER 18/22, 2022 - LOUTRAKI - GREECE

ABSTRACT BOOK

PREFACE

Welcome to the 13th International Congress on Extremophiles!

On behalf of the local organizing committee, we at last are extremely happy to welcome you to Greece, where this congress is taking place for the first time. We would like to thank the International Society for Extremophiles for granting us this opportunity, the big number of national and international institutions, companies and public organizations that made it possible through their generous support, and we look forward to what we know will be a vibrant and inspiring scientific meeting. We are confident that this community, with its strong historic ties, will be able to show, as it has in the past, that building and maintaining strong international collaborative networks is the best way to overcome the difficult and challenging times we all experience.

The conference is taking place at the **Club Hotel Casino Loutraki** from the 18th - 22nd of September 2022, in Loutraki, a seaside resort on the Gulf of Corinth, in Corinthia, Greece. The site is known since antiquity for its natural springs and therapeutic thermal spas, indicated in the names of both the ancient (*Θερμαί/Thermae*; meaning “hot springs”) and contemporary towns (*Loutraki* deriving from the word *Λουτρόν/Loutron*; meaning bath-house or thermae).

Extremophiles 2022 aims to present all the recent updates on basic and applied research on life in extreme environments, from both senior and early-career scientists. The program includes sessions on long-studied aspects such as origin of life, ecology, astrobiology, molecular biology, physiology, ecology and biotechnology. Additionally, we aim to highlight the multiple ways extremophiles can point us to sustainable solutions and help us expand our knowledge on the planetary boundaries during this era of accelerated climate change.

In parallel, the organizers aspire for **Extremophiles 2022** to provide a stimulus for inclusive research interactions, to inspire early career scientists and students through networking with senior and established scientists, and to pave the way for a bright future in this exciting field.

We sincerely thank you for participating in Extremophiles 2022 and we hope you enjoy a stimulating Congress, as well as the famous Greek hospitality and cuisine!

Looking forward to meeting you in Loutraki.
On behalf of the Local Organizing Committee,

Professor Constantinos Vorgias
Congress Chair

Congress chair

Constantinos Vorgias
Biology Department
National and Kapodistrian University of Athens, Greece

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GENERAL INFORMATION ON EXTREMOPHILES 2022

Conference Dates

18th - 22nd of September 2022

Location

Club Hotel Casino Loutraki
https://www.clubhotelloutraki.gr/hotel/
48 Posidonos Str, 20300 Loutraki, Greece
Tel: +302744060300,
E-MAIL: info@clubhotelloutraki.gr

Internet

Wireless Internet connection is freely available in the Congress area.

Liability and insurance

The organizers are not able to take responsibility whatsoever for injury or damage involving persons and property during the congress

Mobile phones, photography and video recording

Participants are requested to keep their mobile phones switched off (or set to silent mode) in the session rooms. Photography and video recordings are strictly prohibited during all scientific sessions.

Time

Eastern European Time

Climate

You can expect an average maximum daytime temperature of around 27°C. Evenings are still pleasantly warm, dropping to around 21°C as an average low temperature during September.

Instructions for presenters

Oral presentations

- KL (Keynote) presentations: 30 minutes (25+ 5 minutes for questions and discussion).
- OS presentations: 15 minutes (12+ 3 minutes for questions and discussion).

The lecture room will be equipped with a PC and a video projector.
Speakers are invited to upload their presentation files (USB memory stick, etc) to the slide point (at the Registration Desk) at the latest by the evening before their scheduled session.
Uploaded files will be available on the lecture room facility.
Speakers are invited to check their presentation on the available PC before any session.
Available presentations system supports ONLY Microsoft WINDOWS and the latest versions of the presentation software.
Mac users are encouraged to save their presentations as “pdf” files.
If the presentation includes any video, the required file format is “avi”.
It is not possible to use a different laptop/devices.

Poster sessions

Posters with odd numbers will be presented during poster session 1, Monday 19th September.
Posters with even numbers will be presented during poster session 2, Wednesday 21st September.

Poster awards

The International Society for Extremophiles (ISE) will honor young researchers with Poster Awards. Candidates of this award are Students, Post Docs and other Non-faculty members. The laureates will be selected by a jury based on the quality and originality of the work and clarity of the poster presentation. The award ceremony will take place before the closing ceremony of the conference on Thursday 22nd September.

Registration and information

The registration desk will be open from 14:30 to 20:30 on Sunday, September 18th, and every day from 08:30 until the end of the sessions.

Name badge policy

Participants (including accompanying persons) are kindly requested to exhibit their congress name badges during all scientific sessions and social events (including Welcome reception and Banquet).

Lunches and coffee breaks

Lunches will be served at the “Deep Blue Restaurant” at Club Hotel Casino.
Access is reserved to ticket holders.
Tickets are included in the participant kit received upon the registration.
Additional tickets can be purchased at the registration desk

Social Events

Welcome Reception: Sunday 18th September at 20:30
Congress Dinner: Wednesday 21st September at 20:00, at the Hotel main Restaurant.
Access is reserved to ticket holders. Tickets are included in your participant kit received at registration.
Excursion by bus to the archaeological site of Mycenae: Tuesday 20th September at 09:00. Access is reserved to ticket holders.

Accompanying persons

Accompanying participants who have registered are welcome to participate in the Welcome Cocktail, Congress Dinner and Social Excursion to Mycenae.

FEMS travel grant awardees

Anja Černoša	*University of Ljubljana, Slovenia*
Alef dos Santos	*Federal University of São Carlos, Brasil*
Seymanur Ersoy Ismir	*University of Economics, Turkey*
Diana Ghevondyan	*Yerevan State University, Armenia*
Valery Lisun	*Institute of the Russian Academy of Sciences, Russia*
Lenka Prochazkova	*Univerzita Karlova v Praze, Czech Republic*
Sulochana Shet	*Goa University, India*

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PROGRAM

SUNDAY 18 SEPTEMBER 2022

from 14:30	Registration
	ROOM A (ROSA & TULIP)
18:00-18:30	**Opening remarks** ***Konstantinos Vorgias and Melina Kerou***
18:30-19:00	**Opening lectures** ***Chair: Christa Schleper***
18:30-19:00	**KL1** Haruyuki Atomi (JPN)
19:00-19:30	**KL2** Bettina Siebers (GER)
19:30-20:00	**KL3** Nikos Kyrpides (USA)
20:30	**Welcome reception**

MONDAY 19 SEPTEMBER 2022

	Room A (Rosa & Tulip)	Room B (Jasmin)
09:00-10:30	**SESSION 1: Microbial defense systems** ***(Chair: Z. Kelman)***	
09:00-09:30	**KL4** Malcolm White (UK)	
09:30-10:00	**KL5** Michael Terns (USA)	
10:00-10:30	**KL6** Erika Wimmer (AT)	
10:30-11:00	Coffee and networking	
11:00-12:00	**SESSION 3: Information Processing systems and Microbial defense** ***(Chairs: I. Zink, M. White)***	
	OS1 Elin Moe (PRT)	
	OS2 Thomas Santangelo (USA)	
	OS3 Isabelle Zink (NL)	
	OS4 José Berenguer (ESP)	
12:00-13:00	**SESSION 2: Microbial physiology** ***(Chair: Bettina Siebers)***	
12:00-12:30	**KL7** Peter Schönheit (GER)	
12:30-13:00	**KL8** Volker Müller (GER)	
13:00-14:30	**Lunch and networking**	
14:30-15:30	**SESSION 4a: Metabolism and Physiology of Extremophiles (I)** ***(Chairs: C. Romão, M. Adams)***	**SESSION 4b: Biotechnology of Extremophiles: Current Uses and Prospects (I)** ***(Chairs: J. Littlechild, E. Parrilli)***
	OS5 Giuliana d'Ippolito (ITA)	**OS9** Ryan Bing (USA)
	OS6 Svenja Höfmann (GER)	**OS10** Anja Černoša (SLO)
	OS7 Mruthyunjay Kubendran Sumathi (USA)	**OS11** Simone De Rose (UK)
	OS8 Popall Rabja (FRA)	**OS12** Anna Florentino (NL)
15:30-18:00	**Poster session with coffee and refreshments (odd numbers)**	
18:00-19:00	**SESSION 5a: Metabolism and Physiology of Extremophiles (II)** ***(Chairs: B. Averhoff, P. Schönheit)***	**SESSION 5b: Sustainable solutions and processes** ***(Chair: M. Kerou)***
	OS13 Frank Robb (USA)	
	OS14 Angela Casillo (ITA)	**OS17** Scott Hamilton-Brehm (USA)
	OS15 Duncan McMillan (NL)	**OS18** Şeymanur Ersoy (TUR)
	OS16 Eunhye Jo (KOR)	**OS19** Ram Karan (SA)
19:00	Free evening / meeting of ISE	

TUESDAY 20 SEPTEMBER 2022

09:00-13:30	Excursion to Archaeological site Mycenae	
13:30-15:00	**Lunch and group photo**	
	Room A (Rosa & Tulip)	**Room B (Jasmin)**
15:00-16:00	**SESSION 6a: Genomics, Metagenomics, Proteomics and Culturomics for Extremophiles (I)** ***(Chairs: E. Peeters, H. Atomi)***	**SESSION 6b: Biotechnology of Extremophiles: Current Uses and Prospects (II)** ***(Chairs: G. d'Ippolito, C. Purcarea)***
	OS20 Antonio Ventosa (ESP)	**OS24** Laura Nissen (GER)
	OS21 Favreau Charly (FRA)	**OS25** Adorjan Cristea (ROU)
	OS22 Elizabeth Watkin (AUS)	**OS26** Parrilli Ermenegilda (ITA)
	OS23 Daniele Daffonchio (SAU)	**OS27** Perugino Giuseppe (ITA)
16:00-16:30	Coffee and networking	
16:30-17:30	**SESSION 7a: Genomics, Metagenomics, Proteomics and Culturomics for Extremophiles (II)** ***(Chairs: B. Siebers, A. Florentino)***	**SESSION 7b: Biotechnology of Extremophiles: Current Uses and Prospects (III)** ***(Chairs: D. Monti, M. Moracci)***
	OS28 Eveline Peeters (BEL)	**OS32** Andrea Strazzulli (ITA)
	OS29 Sreejith Jayasree Varma (GER)	**OS33** Andrea Rodriguez-Sanz (ESP)
	OS30 Alejandra Recalde (GER)	**OS34** Sunita Borkar (IND)
	OS31 Zackary Jay (USA)	**OS35** Samiullah Khan (PAK)

	Room A (Rosa & Tulip)	Room B (Jasmin)
17:30-18:00	Break	
18:00-19:00	**SESSION 8 Microbial Communities & Microbial Ecology at Extreme Conditions (I): Polar and alpine environments** ***(Chairs: D. Cowan, F. Canini)***	
	OS36 Linda Nedbalová (CZE)	
	OS37 Angelina Cordone (ITA)	
	OS38 Fabiana Canini (ITA)	
	OS39 Lenka Prochazkova (CZE)	
19:00	**Free evening**	

WEDNESDAY 21 SEPTEMBER 2022

	Room A (Rosa & Tulip)	Room B (Jasmin)
09:00-10:30	**SESSION 9 Cellular Biology & Evolutionary considerations** ***(Chair: H. Santos)***	
09:00-09:30	**KL9** Shiladitya Dassarma (USA)	
09:30-10:00	**KL10** Sonja Albers (GER)	
10:00-10:30	**KL11** Mirko Basen (GER)	
10:30-11:00	Coffee and networking	
11:00-12:30	**SESSION 10: Deep Subsurface Environments and Piezophiles** ***(Chairs: A. Cario, P. Adam)***	
11:00-11:30	**KL12** Alexander Probst (GER)	
11:30-12:30	**OS40** Peter Fischer (NL)	
	OS41 Sergey Gavrilov (RUS)	
	OS42 Nicolo Ivanovich (SGP)	
	OS43 Samuel Marre (FRA)	
13:00-14:30	**Lunch and networking**	
14:30-15:30	**SESSION 11a: Microbial Communities & Microbial Ecology at Extreme Conditions (II): Hydrothermal environments** ***(Chairs: O. Golyshina, A. Baricz)***	**SESSION 11b: Metabolism and Physiology of Extremophiles (III): Mechanisms of environmental stress resistance** ***(Chairs: S. Albers, M. Basen)***
	OS44 Ema Kostešić (HRV)	**OS48** Reinier Egas (NL)
	OS45 Bernardo Barosa (ITA)	**OS49** Masahiro Ito (JPN)
	OS46 Costantino Vetriani (USA)	**OS50** Takayuki Ohira (JPN)
	OS47 Anastasia Galani (NL)	**OS51** Célia V. Romão (PRT)
16:00-18:00	**Poster session with coffee and refreshments (even numbers)**	
18:00-19:00	**SESSION 12a: Microbial Communities & Microbial Ecology at Extreme Conditions (III)** ***(Chairs: C. Schleper, A. Ventosa)***	**SESSION 12b: Genomics, Metagenomics, Proteomics and Culturomics for Extremophiles (III)** ***(Chairs: E. Watkin, A. Probst)***
	OS52 Don Cowan (SA)	**OS56** Guillaume Tahon (NL)
	OS53 Olga Golyshina (UK)	**OS57** Jessica Downing (UK)
	OS54 Raeid Abed (OMN)	**OS58** Juan Gonzalez (ESP)
	OS55 Andreea Baricz (ROM)	**OS59** Raffaella Margherita Zampieri (ITA)
20:00-22:30	**Greek Gala Dinner**	

THURSDAY 22 SEPTEMBER 2022

	Room A (Rosa & Tulip)	Room B (Jasmin)
	late start to allow for checkout	
09:30-11:00	**SESSION 13: Extremophiles: Link Between Earth and Astrobiology** ***(Chairs: A. Kish, E. Chatzitheodoridis)***	
09:30-10:00	**KL13** Elias Chatzitheodoridis (GRE)	
10:00-11:00	**OS60** Adrienne Kish (FRA)	
	OS61 Oddur Vilhelmsson (ISL)	
	OS62 Kristina Beblo-Vranesevic (GER)	
	OS63 Donato Giovannelli (ITA)	
11:00-11:30	Coffee and networking	
11:30-12:00	**Closing lecture** ***Chair: Konstantinos Vorgias***	
	KL14 Marco Moracci (ITA)	
12:00-12:30	Garabed Antranikian (GER) - Closing remarks	
12:30-13:00	Poster Awards Ceremony	
13:00	**Closing Ceremony**	

KEYNOTE PRESENTATIONS

New players in FtsZ-based cell division in archaea

Phillip Nußbaum and Sonja-Verena Albers

In most bacteria, cell division depends on the tubulin homolog FtsZ and other proteins that form a large complex termed the divisome. This complex is composed out of many proteins that together ensure proper execution of cell division resulting in two daughter cells of same size and DNA content. Also many archaea involve FtsZ homologs for cell division but beside these homologs and the FtsZ membrane anchor SepF no other proteins being part of their cell division system have been identified. Here, we demonstrate that three homologous proteins, all composed of a single conserved protein domain, partially play important roles in cell division in *Haloferax volcanii*. These proteins were identified by immunoprecipitation experiments using HA tagged SepF and are from now on termed CdpC1-3 (cell division protein C). Fusion of a C-terminal mNeongreen tag to the CdpC proteins revealed their localization at the cell center, implicating a role in cell division. Moreover, it was not possible to delete CdpC1 and depletion of the protein resulted in strongly deformed cells assigning the protein an essential role in the cell. In contrast the genes coding for CdpC2 and CdpC3, were successfully deleted. While the *cdpC2* deletion showed an aberrant cell shape and reduced viability, the *cdpC3* deletion strain remained unaffected. Interestingly, the formation of an intact FtsZ1 ring in the Δ*cdpC2* strain was hampered whereas formation of the FtsZ2 and SepF-ring remained normal. Additionally, localization of all three CdpC proteins depend on the presence of SepF since during SepF depletion the ring like localization pattern of the CdpCs changed to single foci or a completely diffuse signal.

The pentose bisphosphate pathway and related enzymes and pathways in archaea

Haruyuki Atomi, Yuta Michimori and Takaaki Sato

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The pentose bisphosphate pathway directs the ribose moieties of nucleosides to central carbon metabolism. The pathway was identified in the hyperthermophilic archaeon *Thermococcus kodakarensis*,[1] and until now, seems to be specific to Archaea. The enzymes constituting the pentose bisphosphate pathway in *T. kodakarensis* are nucleoside phosphorylases, ADP-dependent ribose-1-phosphate (R1P) kinase, ribose-1,5-bisphosphate (R15P) isomerase and ribulose-1,5-bisphosphate (RuBP) carboxylase/ oxygenase, or Rubisco. A nucleoside-5'-monophosphate (NMP) phosphorylase is also present and provides a metabolic link between the pathway and NMPs.[2] R1P kinase, R15P isomerase, and NMP phosphorylase catalyze reactions that had not been previously recognized in biology. Genome sequences suggest that the enzymes of the pentose bisphosphate pathway from *T. kodakarensis* are widely conserved throughout members of the Thermococcales.
Concerning other groups of Archaea, we noticed that a large portion of halophilic archaea possess R15P isomerase homologs. Among these species, although many also harbored Rubisco homologs, a significant number did not. Comparative genomic and biochemical analyses suggested the presence of a unique variant of the pentose bisphosphate pathway in these halophiles, which we designate the non-carboxylating pentose bisphosphate pathway.[3] In Crenarchaea, a protein that displays ATP-dependent R1P kinase activity was identified, but proteins responsible for the conversion of R15P are still unknown.[4] Although the function of the carboxylase activity of Rubisco in *T. kodakarensis* is now understood, how the organism deals with the oxygenase activity of Rubisco was not known. Recent studies indicate the presence of a salvage pathway for 2-phosphoglycolate in *T. kodakarensis*, the product of the oxygenase activity of Rubiscos.[5]

References

1. Aono et al. Nat. Chem. Biol., 2015.
2. Sato et al. Science, 2007.
3. Sato et al. See poster presentation.
4. Aziz et al. J. Bacteriol., 2018.
5. Michimori et al. See poster presentation.

Tracing the Origins of Life? Laboratory evolution of a thermophile towards a lower growth temperature

Mirko Basen, Christoph Prohaska, Erik Zschaubitz, Antonia Friedrichs and Maria Lehmann

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Life on Earth may have originated at moderate hydrothermal vents with the Last Universal Common Ancestor (LUCA) putatively thriving on hydrogen and carbon dioxide, as an autotrophic thermophilic anaerobe [1]. Thermophily may thus have been ancient, and the adaptation to moderate temperatures the derived phenotype – opposite to what is usually discussed in literature. Experimentally, laboratory evolution of thermophiles to lower optimal growth temperatures has not been addressed. In my talk I will describe our strategies and efforts to adapt thermophilic microorganisms to lower growth temperatures. We focus mainly on *Thermoanaerobacter kivui,* the most thermophilic acetogenic bacterium described, with an optimal growth temperature (T_{OPT}) of 66 °C [2]. Its physiology possibly resembles that of LUCA, making it a perfect model organism to study the evolutionary changes necessary to grow at moderate temperatures. We carried out growth experiments at suboptimal growth temperatures and found that *T. kivui* is able to grow at a temperature of 39 °C, more than 20 °C below its T_{OPT}. Subsequently, we followed different strategies to possible obtain individual cells better adapted to lower temperatures, including the selection of large colonies grown in agar at lower temperatures and adaptive laboratory evolution (ALE) at 45°C. One promising isolate (Iso50) and a culture with a shift in its T_{opt} from 66°C to 60°C were obtained and characterized. The phenotypic changes as well as single nucleotide polymorphisms in the adapted strains gave first ideas of the required alterations needed for growth of *T. kivui* at suboptimal temperatures, which may resemble a step in early evolution.

References

1. Weiss et al. (2016). Nat. Microbiol. **1**:16116.
2. Basen and Müller (2017). Extremophiles. **21**:15-26.

Methods and techniques for biosignature detection in astrobiology

Elias Chatzitheodoridis[1,2]

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Biosignatures [1] are chemical or morphological indicators [2,3] of living organisms, extinct or extant. They are of paramount importance to astrobiology, especially when extinct organisms must be identified in extraterrestrial environments. Special, often extreme environments on early Earth are also important to elucidate how life has originated on our planet, through the relation of the first extremophilic microorganisms and the ancient environments. Missions to Mars focus their exploration activities on identifying biosignatures, eventually proving that life has also existed on other planetary bodies. New missions to Venus are also designed to perform similar research on the planet's surface and its atmosphere. Recently, biosignature research has be expanded to exoplanets, *i.e.*, searching for evidence of living organism from signatures in the atmospheres of exoplanets.

Here, we will start with a brief review of the topic, and we will elaborate on existing techniques and methods to identify biosignatures [4]. Apart from the chemical biosignatures, we will provide examples of textural biosignatures, but we will also describe morphological structures that do not constitute true biosignatures [5], referred to as pseudo-biosignatures.

We will also describe powerful techniques and methodologies [6] we use in the detection of chemical and molecular biosignatures, such as Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) [7], Laser Desorption Mass Spectrometry (LDMS), and Raman [8].

We will finally describe new projects, such as the OxR project [9], aiming to build microfluidic systems that are capable of detecting whether extraterrestrial regoliths, soils, and ices are appropriate to be explored for molecular biosignatures, and therefore, if they should be sampled by the currently running sample return missions, such as NASA's Mars2020 mission and its Perseverance rover. OxR detects reactive oxygen species (ROS) through the detection of gas oxygen released when regolith reacts with water. The concept dates back to the Viking 'Gas Exchange Experiment' (GEX) [10], where the mixing of Martian soil with nutrient material brought from Earth released significant amounts of gaseous oxygen, however, not released from the metabolism of living microorganisms.

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2. Cady S.L., Farmer J.D., Grotzinger J.P, Schopf J.W., Steele A. (2003). *Astrobiology*, **3**, 351–368.
3. Broz A.P. (2020). Life **10**, 113. https://doi.org/10.3390/life10070113
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Comparative genomics and physiological capabilities of Haloarchaea

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Halophilic Archaea (Haloarchaea) are ubiquitous in hypersaline environments on Earth. In addition to saturating concentrations of sodium chloride, such environments may be subject to desiccation, toxic ions, anaerobic and microaerobic conditions, temperature extremes, intense sunlight, and radiation. A decade after sequencing of the genome of *Halobacterium* sp. NRC-1 in 2000, we conducted comparative genomics against a dozen other haloarchaea including the cold-adapted *Halorubrum lacusprofundi* from Antarctica. Similar studies have now been extended using recent isolates from the Great Salt Lake of Utah, Dead Sea of the Middle East, and Salar de Uyuni and Tarija salt mine of Bolivia. These studies enhance our understanding of haloarchaeal life in multiple extremes, including their conserved orthologous and signature genes, highly acidic proteins, oxygen-free respiration capabilities, efficient DNA repair systems, light-shielding pigments, diverse retinal photoproteins, and buoyant gas-filled proteinaceous nanoparticles, several of which are valuable for biotechnology and biomedicine. Haloarchaea have evolved remarkable characteristics to survive and prosper in their harsh environment despite multiple stressors, providing valuable clues on how life may potentially survive on other worlds.

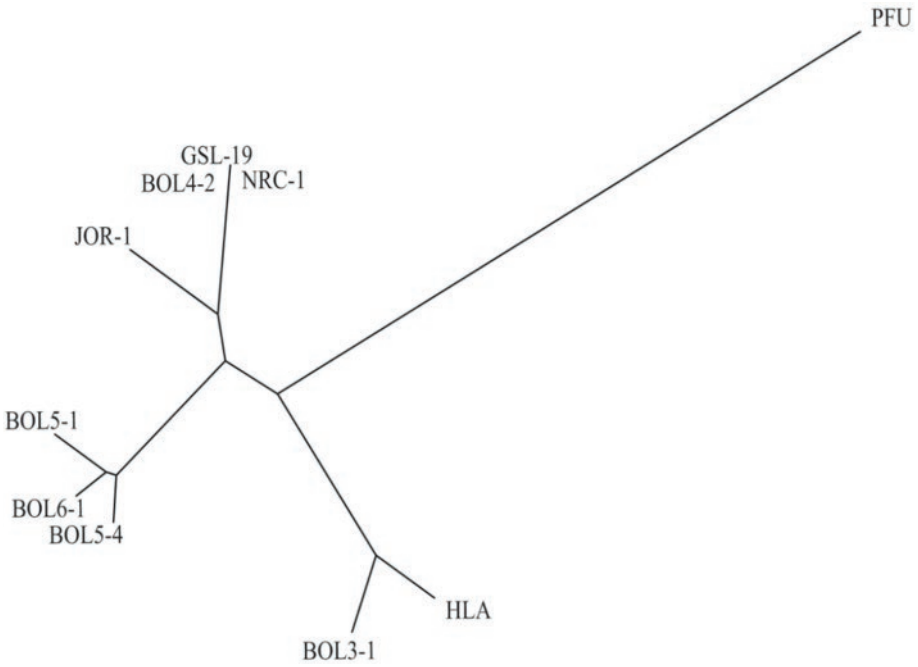

Figure showing 16 rRNA NJ tree of diverse Haloarchaea.

Microbiome Data Science: from the Earth Microbiome to the Global Virome

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Microbiome research is rapidly transitioning into Data Science. The unprecedented volume of microbiome data being generated pose significant challenges with respect to standards and management strategies, but also bear great new opportunities that can fuel discovery. Computational analysis of microbiome samples involving previously uncultured organisms, is currently advancing our understanding of the structure and function of entire microbial communities and expanding our knowledge of genetic and functional diversity of individual micro-organisms and their interplay in the communities. I will describe some of our approaches and will emphasize the value of big data integration in enabling the exploration of large metagenomic datasets and the discovery of novelty. I will present current approaches and will discuss a few science vignettes in the exploration of microbial, viral, and functional diversity.

Exploring the biodiversity of an extreme environment: discovery of novel enzymes, metabolisms and biotechnology applications

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Terrestrial hot springs have been sought out by biochemists, microbiologists, geochemists, and astrobiologists around the globe who are interested in their chemical properties, which provide a strong selective pressure on local microorganisms. Microbes in these communities have evolved strategies to thrive in these conditions by converting hot spring chemicals and organic matter into cellular energy. The advent of metagenomics allowed access to an enormous amount of sequencing data, but the functional assignment of the genes is still hampered by long and laborious microbiological and biochemical studies.

The Pisciarelli hot springs (Naples, Italy), whose access for studies is relatively simple, have long-standing popularity in the scientific community being the site where *Sulfolobus (Saccharolobus) solfataricus*, a workhorse for the enzymology and molecular biology of Archaea has been isolated for the first time[1]. We report here on recent metagenomic studies on Pisciarelli solfatara documenting the continuous evolution of the site and how the microbial diversity reflects the recent geothermal activity[2, 3]. How (meta)genomic analysis helps in the discovery of novel enzymes, their assignment to novel metabolic functions, and to biotechnology applications[4, 5], will be also discussed.

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Electrons in the fast lane: Membrane-anchored HDCR nanowires drive hydrogen-powered CO_2 fixation

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Acetogenic bacteria are characterized by a special pathway for CO_2 fixation, the Wood-Ljungdahl pathway. The first enzyme in the methyl branch of this pathway reduces CO_2 to formate. The enzymes involved are formate dehydrogenases that use reduce ferredoxin or NADPH as reductant, some species also use electron bifurcation catalyzed by two consecutive enzymes, a FeFe hydrogenase and a formate dehydrogenase that are connected by the soluble electron carriers NAD(P) and ferredoxin. The thermophilic acetogenic bacterium *Thermoanaerobacter kivui* has a special enzyme that directly couples oxidation of H_2 by a FeFe hydrogenase to CO_2 reduction by a formate dehydrogenase without soluble intermediates; instead, the hydrogenase (HydA) and the formate dehydrogenase (FdhF) are directly linked to each other by two small, FeS-containing proteins (HycB3 and HycB4) (1). This hydrogen-dependent CO_2 reductase (HDCR) forms long filaments that enhance CO_2 fixation (2); actually, the HDCR from the thermophile has the highest ever reported rates for CO_2 reduction to formate (3). The structure of a short HDCR filament wwas revealed by cryo-electron microscopy. The minimum repeating unit is a hexamer consisting of a formate dehydrogenase (FdhF) and two hydrogenases (HydA2) bound around a central core of one HycB3 and two HycB4. These small bacterial polyferredoxin-like proteins oligomerize *via* their C-terminal helices to form the backbone of the filament. Structure-directed mutagenesis with enzymatic analysis showed that filamentation and rapid electron transfer through the filament enhances HDCR activity. Cryo-electron tomography revealed that HDCR filaments bundle into large ring-shaped superstructures attached to the plasma membrane. This supramolecular organization may further enhance HDCR stability and connectivity to form a specialized metabolic subcompartment within the cell (4). The outstanding catalytic activities of the bidirectional enzyme make the HDCR a prime candidate for a biocatalyst to store hydrogen, a promising alternative to fossil fuels in (bio)technology. We developed a system with a mesophile that allows multiple cycles of reversible hydrogenation of CO_2 to formic acid in a single bioreactor. The process was kept running over two weeks producing and oxidizing 330 mM formic acid in sum. Unwanted side product formation in form of acetic acid was prevented through metabolic engineering of the organism (5). The demonstrated process design can be considered as a future “bio-battery” for the reversible storage of electrons in form of H_2 in the versatile compound formic acid.

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Ecophysiology of uncultivated archaea from the deep biosphere

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Recent advances in environmental genomics have highlighted how little we actually know about the uncultivated majority of extremophilic microbes, i.e. bacteria, archaea, and their viruses. Although these microbes may play crucial roles in global ecosystem functioning, we have little information about their physiology, and studies of such in ecosystems remains a general gap of knowledge. Here, I summarize our past and present research endeavours of studying the ecophysiology of Candidatus Altiarchaea [1], which can dominate deep subsurface ecosystems under high CO_2 pressure [1,2,3], and their associated viruses [4]. Ca. Altiarchaea employ a modified Wood-Ljungdahl pathway for carbon fixation [1] and thus act as primary producers of the deep biosphere. Their unique cell morphology includes cell surface appendages called *hami*, and a double membrane enabling accurate identification in electron microscopy [1,4,5]. Using genome-resolved metagenomics coupled to virusFISH we identified novel archaeal viruses that infect these archaea [4] and studied host CRISPR system diversification cross space and time. Interestingly, virocells of Candidatus Altiarchaea show a strict cellular organization with a separation of host DNA and viral genome, the latter co-localized with host ribosomes for viral biogenesis. We also solved a major bottleneck in genomics research of Ca. Altiarchaea, which usually resulted in extremely fragmented genome reconstruction. Using an elegant way of Oxford Nanopore Technology-based metagenomics and short-read shotgun sequencing for error correction we reconstructed the first complete Ca. Altiarchaea genome. Its genome architecture is dominated by globally highly conserved core genes, multiple transposable elements and horizontal gene transfer [3] likely creating microdiversity and genome fluidity. Our results are an unprecedented example of ecophyiological studies of uncultivated extremophilic microbes and shed light on genome evolution in the deep biosphere.

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New Views on an Old Enzyme: Allosteric Regulation and Evolution of Archaeal Pyruvate Kinases

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Pyruvate kinases (PKs) catalyse the final step of glycolytic pathways in all three domains of life. PKs from most bacteria and eukarya are allosterically activated by sugar phosphates, mostly fructose-1,6-bisphosphate (FBP) or AMP. In the domain of archaea only few PKs were analyzed and found not to respond to the classical allosteric effectors. Recently, we identified 3-phosphoglycerate (3PG) as a novel allosteric activator of the PK from the hyperthermophilic archaeon Pyrobaculum aerophilum, a member of Thermoproteales [1]. Here we report a comprehensive analysis of PKs from most archaeal groups with respect to their allosteric regulation by 3PG and sugar phosphates. Allosteric activation by 3PG appears to be restricted to PKs from Thermoproteales. FBP-regulated PKs were not found in archaea. PKs from hyperthermophilic Methanoarchaea, e.g. Methanocaldococcus jannaschii, were activated by AMP. Phylogenetic analyses indicate that PKs originated in hyperthermophilic archaea and then evolved in two distinct lineages according to their different modes of allosteric activation. One lineage is activated by sugar phosphates, and the other by the novel effector 3PG [2].

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Adaptation to high temperature from a metabolic point of view

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Sulfolobus acidocaldarius and *Saccharolobus solfataricus* are thermoacidophilic members of the Crenarchaeota with heterotrophic growth at pH 2-3 and 80°C. In recent years, we have been able to identify the degradation pathways for various carbohydrates for energy production as well as gain insights into the function of carbohydrates in the cell. Nevertheless, we are only at the beginning to understand the basic features of archaeal metabolism and its regulation[1].

In general, the text-book picture of a perfect, well organized metabolism with highly specific enzymes has changed. Promiscuous enzymes and non-catalyzed reactions challenge metabolism and require an elaborate repair and rescue system. This, so-called 'underground metabolism', is a special challenge for hyperthermophilic Archaea that thrive at temperatures about 80 °C and possess modified central metabolic pathways often with promiscuous enzymes. Hence, the question arises how extremely thermophilic Archaea can operate their unusual metabolism at temperatures where many pathway intermediates are unstable? Our current insights into the function of carbohydrates in *Sulfolobus* species and different strategies to adapt to enhanced underground metabolism at high temperature will be discussed[2].

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Archaeal histones specify the site of foreign DNA integration at CRISPR loci

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CRISPR systems confer adaptive immunity against viruses and other mobile genetic elements to a broad range of archaeal and bacterial species. CRISPR systems adapt to invasive mobile genetic elements by incorporating short fragments of foreign DNA (referred to as spacers) into the CRISPR array of the host. Remarkably, new spacers are incorporated into CRISPR loci in a directional manner with integration almost invariably taking place at the repeat sequence located immediately downstream of the leader DNA rather than at identical repeats found throughout the CRISPR array. *In vitro*, we and others have shown that Cas1-Cas2 integrase complexes often integrate spacers indiscriminately at each repeat of the CRISPR array, suggesting that host factors normally ensure that new spacers are added selectively at the leader proximal repeat *in vivo*. I will present exciting findings supporting a key role for histones in directing spacer integration in archaea. We observe histones binding to leader DNA of *Pyrococcus furiosus* CRISPR arrays *in vivo* and show that new spacer integration is inhibited in strains lacking one of the two histone proteins. Moreover, we demonstrate that purified histones are sufficient to direct spacer integration to the leader-proximal repeat *in vitro*. Our results indicate that histones coordinate the formation of temporally-ordered and heritable immunological memory used to prevent recurrent infections by archaeal viruses and plasmids.

Cyclic Nucleotide Signalling in virus:host conflict

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Prokaryotic cells utilise a diverse array of cyclic nucleotide second messengers to coordinate defence against invading mobile genetic elements (MGE). Type III CRISPR systems detect foreign RNA and generate cyclic oligoadenylate (cOA) second messengers that activate powerful degradative nucleases, while CBASS (Cyclic nucleotide based antiphage signalling systems) make a wide range of cyclic di- and tri-nucleotides that frequently activate abortive infection (cell death) pathways. This talk will focus on the mechanisms of cyclic-nucleotide signalling in anti-viral defence, focussing on two examples under study in our lab. The first is a cOA degrading “ring nuclease” from the thermophile *Archaeoglobus fulgidus (1).* The second is a bacterial CBASS effector which is activated by cOA, degrading cellular NAD+ with an enzymatic TIR domain (2). Both enzymes use the cOA molecule as a “glue” to build large protein machines that activate the defence systems in response to viral invasion.

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Compelling cellular reactions of *Saccharolobus solfataricus* upon deletion of a type III CRISPR-Cas system

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Saccharolobus solfataricus, a thermoacidophilic member of the Thermoproteia (formerly Crenarchaeota), was originally isolated from acidic hot springs and grows optimally at a temperature of 78°C and pH 3. Sharing its natural habitat with many different viruses, *S. solfataricus* exhibits extensive CRISPR-Cas (Clustered regularly interspaced short palindromic repeats - CRISPR associated proteins) systems, which were previously shown to act as RNA-guided adaptive immune systems in prokaryotes. While most other CRISPR-Cas systems specifically recognize DNA targets, type III CRISPR-Cas systems target nascent viral RNA transcripts. *S. solfataricus* harbors two type III CRISPR-Cas systems which, based on their interference mechanism, hold the potential to regulate cellular processes at the post-transcriptional level. There are indeed some indications for additional cellular roles of these enzymatic complexes apart from virus defense. In this study, we deleted a type III CRISPR-Cas module in *S. solfataricus* in order to discern its putative functional links to other cellular processes and alternative roles besides immunity. Notably, the knockout mutants grew considerably faster compared to the wildtype strain. Effects of the genomic deletion of the type III interference module on the transcriptional landscape were analyzed using RNAseq. Interestingly, we found a significant upregulation of genes associated with energy production and conversion, including complex I of the respiratory chain, as well as carbohydrate or amino acid transporters. Generally, a significant accumulation of genes encoding transmembrane proteins in the upregulated proportion of the transcriptome suggests interconnections between the type III CRISPR-Cas system and various membrane-associated processes. Investigations on the underlying mechanisms leading to this phenotype are currently under way.

ORAL PRESENTATIONS

Metabolic Activity of Microorganisms in Microbial Mats Thriving at Saturation-level Salinities and Their Potential Use in Biofuel Production

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Hypersaline microbial mats are dense microbial ecosystems capable of performing complete element cycling and are considered analogs of Early Earth and hypothetical extraterrestrial ecosystems. We studied the functionality and limits of key biogeochemical processes, such as photosynthesis, aerobic respiration, and sulfur cycling in salt crust-covered microbial mats from a tidal flat at the coast of Oman. We measured light, oxygen, and sulfide microprofiles as well as sulfate-reduction rates at salt saturation and in flood conditions and determined fine-scale stratification of pigments, biomass, and microbial taxa in the resident microbial community.

The salt crust did not protect the mats against irradiation or evaporation. Although some oxygen production was measurable at salinity ≤ 30% (w/v) in situ, at saturation-level salinity (40%), oxygenic photosynthesis was completely inhibited and only resumed two days after reducing the pore water salinity to 12%. Aerobic respiration and active sulfur cycling occurred at low rates under salt saturation and increased strongly upon salinity reduction. Apart from high relative abundances of Chloroflexi, photoheterotrophic Alphaproteobacteria, Bacteroidetes, and Archaea, the mat contained a distinct layer harboring filamentous Cyanobacteria, which is unusual for such high salinities.

Our results show that the diverse microbial community inhabiting this saltflat mat ultimately depends on periodic salt dilution to be self-sustaining and is rather adapted to merely survive salt saturation than to thrive under the salt crust.

Turning blue: suboptimal temperature triggers the synthesis of dark-blue pigment in the moderately halophilic *Chromohalobacter marismortui*

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Chromohalobacter marismourtui is a moderately halophilic bacterium that was reported to produce a dark-blue pigment when cultured at suboptimal temperature (23°C) on a glycerol-based medium [1]. Ever since the nature of the pigment remained elusive. In this study, we aimed to elucidate the chemical nature of this cryptic dark-blue pigment produced by *C. marismortui*. The type strain DSM 6770 grows optimally at 37°C without producing the pigment. Pigment production was triggered when the growth temperature ranged between 4-30°C on both glucose and glycerol supplemented media. The pigment was extracted in distilled water by sonication, washed, solubilized in DMSO and analysed by VIS-spectrophotometry, EI-MS, ^{1}H-NMR, and Raman spectroscopy. The peak in the absorbance spectrum was at 610 nm whereas the EI-MS indicated a compound with m/z=248. The ^{1}H-NMR and Raman analyses have confirmed that the pigment belongs to the pyridone-based compounds and is related to the indigo dye. Further investigations are required to understand the role of the pigment during suboptimal growth and to elucidate the role of the pigment in cellular physiology during growth at suboptimal temperatures.

Acknowledgements: Support received from Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project numbers PN-III-P4-ID-PCE-2016-0303 and PN-III-P4-ID-PCE-2020-1559.

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Microbial diversity and salt stress response strategies in uncultured lineages from the organic-rich sediments of Romanian saline lakes

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The salt stress response in prokaryotes involves well-conserved active mechanisms: import or biosynthesis of compatible solutes or import of potassium cations in the `salt-in` osmoadaptive strategy. Based on genome-resolved metagenomic data we aim at exploring osmoadaptation in candidate phyla inhabiting organic-rich, brackish-to-hypersaline sediments sampled from Amara (1.1% salinity), Tekirghiol (11.1% salinity) and meromictic Ursu (42.8% salinity) lakes. Of the 467 metagenome-assembled genomes (MAGs) recovered, 173 were assigned to uncultured archaeal (59) and bacterial (114) lineages. Our analyses indicate that *Hadarchaeota* (encoding multicomponent Na^+:H^+ antiporters), *Iainarchaeota*, *Micrarchaeota*, *Nanoarchaeota* and *Nanosalinia* (encoding Trk potassium uptake system) might mainly rely on `salt-in` strategy. Import and biosynthesis of compatible solutes (glycine betaine, trehalose, ectoine) are osmoadaptive traits predominantly found in *Lokiarchaeota* and *Bathyarchaeota*. Multicomponent Na^+:H^+ antiporters were inferred in *Bipolaricaulota*, *Cloacimonadota*, *Caldatribacteriota*, *Defferibacterota*, *Fermentibacterota*, *Krumholzibacteriota*, *Margulisbacteria*, *TA06_A*, *UBP7_A*, *WOR-3_B*. OpuA-D compatible solute import system was detected in *Bipolaricaulota* and *Cloacimonadota*. Genes for ectoin biosynthesis were detected in *Caldatribacteriota*, *Cloacimonadota*, *Defferibacterota*, *Fermentibacterota*, and *Hydrogenedentota*. *Margulisbacteria* show potential for ectoine, proline, and trehalose biosynthesis. An overall trend towards the predominance of the `salt-in` strategy in sediments with highest salinity was noted across recovered uncultured archaeal and bacterial MAGs.

Keywords: uncultured lineages, organic-rich saline sediments, osmoadaptation, Archaea, Bacteria.

Acknowledgements: Support received from Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project number PN-III-P4-ID-PCE-2020-1559.

Shallow-water hydrothermal vents of Italy as windows to the relationships between the Geosphere and Biosphere

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Shallow-water hydrothermal vents (SWHVs) are unique marine environments. As a result of their proximity to the surface environment, SWHVs are strongly influenced by solar energy. In contrast with their deep-sea counterparts, in these systems, the primary production is reliant upon a mixture of phototrophy and chemolithoautotrophy[1]. Arc-hosted shallow-water vents, such as the ones found on the coast of Italy, are characterized by a dynamic fluid geochemistry[2], making them the ideal locations to study the intricate relationships between the Geosphere and the Biosphere. Here, we present the combined results on the geochemistry and microbiology of shallow-water vents of the Aeolian archipelago (Sicily) and the Gulf of Naples (Campania) through the combination of 16S rRNA amplicon sequencing and geochemical approaches. Given the diverse volcanic and tectonic settings where these vents occur, we explored how geochemical differences shape the microbial communities and the role of these ecosystems on the local biogeochemical cycles. Most importantly, due to the ecological relevance of shallow-water hydrothermal vents to the surrounding marine environment, we argue for the inclusion of these unique environments into current and future conservation efforts.

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New modelorganisms for astrobiology from mars analog environments

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A selection of the core questions in astrobiology deal with the origin of life on Earth, life in extreme environments on Earth, and the search for past and present life on other celestial bodies. We are therefore searching for new model-organisms for astrobiology in extreme environments, the so-called Martian analog environments, which are similar to past and present-day Mars in some characteristics and properties (anoxic conditions, low nutrient availability, high salinity, low temperatures, etc.).
At the moment we are working with three facultative anaerobic model-organisms, namely *Yersinia intermedia* MASE-LG-1, *Buttiauxella* sp. MASE-IM-9, and *Salinisphaera shabanensis*. These organisms are being evaluated for their tolerance to Mars relevant stress factors such as desiccation, Martian atmosphere, radiation (polychromatic / monochromatic UV; ionizing radiation), oxidizing compounds (perchlorates), and the presence of an analog Martian regolith. All these influencing factors were tested under anoxic conditions as single stresses and in combination [1, 2].
The results showed that the new model-organisms for the most part clearly survived the various stress factors, thus qualifying them as possible candidates for our future space experiment called MEXEM (Mars EXposed Extremophiles Mixture). MEXEM which will be an exposure experiment which is installed on the outside of the international space station.

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Barriers against extracellular DNA in *Thermus thermophilus*

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Thermus thermophilus HB27 encodes a very active natural competence apparatus (NCA)[1] that participates in both the acquisition of extracellular DNA[2] and the internalization of DNA donated by a conjugation mate[3]. The high efficiency of this NCA system, its constitutive expression, and the diversity of eDNA origins that can be acquired, forces the organism to keep and adequate balance between acquisition of new capabilities and defence against putatively malicious eDNA. As a result of this equilibrium, a panoply of naive and trained defence barriers are encoded in the genome of *T. thermophilus* HB27. The argonaute protein constitutes the best known example of naive defence system, acting as a barrier against eDNA by DNA-DNA interference[4], but also other proteins like a DNA primase polymerase (Ppol), encoded by a mobile integrative element (ICETh2), are also relevant as eDNA barriers through not yet defined mechanisms[5]. In this communication, we identify the excinuclease AddAB as an additional barrier against the acquisition of eDNA. We show that mutants defective in this enzyme increase their transformation efficiency in three orders of magnitude. A functional equilibrium between AddBA and Ppol activity in the regular DNA repair functions of the cell is proposed on the basis of the frequent spontaneous null mutations in the *addBA* genes that appear in mutants lacking the Ppol protein.

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Funding information
This work has been funded by project PID2019-109073RB-I00 from the Spanish Ministry of Science and Innovation.

Leveraging Extreme Thermophily for Enhanced Plant Biomass Conversion to Bio-based Chemicals and Fuels

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Microbial degradation and conversion of plant biomass into industrial products has been investigated as an alternative to fossil-derived chemicals. Consolidated bioprocessing aims to deconstruct and convert plant biomass in a single step. Certain extremely and moderately thermophilic bacteria (Such as *Caldicellulosiruptor bescii* and *Acetivibrio thermocellus*) stand out has major candidates for this process due to their native ability to deconstruct complex (hemi)cellulolytic substrates without pretreatments [1]. Production of valuable products in these microbes at industrially relevant levels still remains a barrier to commercial use, although production of several commercial chemicals has been demonstrated, including acetone, butanol, and ethanol [2,3,4].

Extreme thermophily offers significant advantages compared to mesophilic and moderately thermophilic counterparts, allowing for novel product separation strategies and increased resistance to contamination. Here, we will present work that investigates and exploits these advantages. In particular, we will present further progress in engineering *C. bescii* to produce volatile chemicals (acetone, ethanol) at extreme temperatures (>70°C) to enable *in situ* separation of desired products from fermentation headspace (dubbed 'bioreactive distillation') [5]. We also define a thermophilic threshold for resistance to contamination from non-pretreated plant biomass.

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Production, optimization and partial characterization of alkali-stable alpha amylases from *alkalihalobacillus* sp. Strains sb-d and sb-w

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Alkali-stable amylases offer greater advantages over other amylases in biotechnological applications. In the present study we aimed to produce, optimize and characterize amylase from *Alkalihalobacillus* sp. strains SB-D and SB-W isolated from sediment samples of an agrochemical factory. The strain SB-D efficiently hydrolyzed starch with optimized conditions of 2% inoculum, pH 10.3, 25°C, at 200 rpm after 24 h, while for SB-W it was 2% inoculum, pH 10.3, 55**°C, at 250 rpm and after 16 h. Further, crude amylases from strain SB-D and SB-W were partially purified to 2.9 and 1.8 fold, respectively by ammonium sulphate precipitation method. SDS-PAGE revealed protein bands from 290 to 12.9** kDa in partially purified amylase of both the strains. Interestingly, amylase from SB-W retained 80% activity in the presence of butanol, isopropanol and ether and showed activity at broad pH range of 7.3-11.3 and temperature (25-75˚C). Starch hydrolyzing enzyme from both the strains belonged to α-amylase, confirmed using reducing sugar and iodine blue value similar for commercial α-amylase. Further, amylases showed strict requirement of metal ions for their activity and were stable in the presence of detergent additives. Conclusively, SB-D and SB-W are potential producers of alkali and detergent stable amylases of biotechnological significance.

Keywords: Agrochemical factory, *Alkalihalobacillus*sp.strain SB-D, *Alkalihalobacillus* sp.strain SB-W, Alkali stable, Organic solvent tolerant .

Influence of rocks colonization on bacterial diversity in soils of Antarctic deserts

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The McMurdo Dry Valleys (MDVs), the widest permanently ice-free area of Antarctica, are considered the coldest and driest desert on Earth, also characterized by strong winds and UV irradiation regimes. These conditions make MDVs one of the closest terrestrial analogues of the Martian environment[1]. Despite many studies have been carried out until now on soil diversity in these environments, many questions are still open. Among these, the potential contribution of rock-dwelling communities to soil diversity has never been clarified. Indeed, biological colonization of rocks induces surface fragments detachment and dispersal, many of which fall into the soils[2]. To answer this question, soil samples were collected in three different localities as close as possible to endolithically colonized sandstone outcrops and at increasing distances (50 and 100 meters). Samples of the corresponding rock outcrops were collected as well. Bacterial diversity has been investigated through a metabarcoding approach, targeting the 16S rDNA gene. Because it is still unclear whether the life forms identified by molecular approaches are present as living organisms or as extracellular DNA from dead cells[3], samples were also treated with PMA (Propidium Mono-Azide), which binds to extracellular DNA and inhibits its amplification, and subjected to metabarcoding screening. Data on the diversity and distribution of soil bacteria, in comparison to the rock communities, will be presented. Additionally, to give insights into the drivers of life establishment in this environment, the diversity and composition of communities will be correlated with physicochemical parameters such as carbon and nitrogen content, pH, moisture, cation exchange capacity, and soil granulometry.

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The acetyl-rich capsular polysaccharide isolated from the vesiculating psychrotolerant bacterium *Shewanella vesiculosa* HM13

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Cold-adapted Gram-negative bacteria produce Extracellular Membrane Vesicles (EMVs), small spheres (20–250 nm) released by the bacterial membrane and comprising phospholipids, lipopolysaccharides (LPSs), proteins, peptidoglycans, DNA and RNA. [1,2] 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 the EMVs have been performed. [3] However,

while the exact function of these nanoparticles has been extensively investigated in pathogens, it is still largely underexplored in the marine and cold environment.[4] In addition, the physicochemical properties of these vesicles have been poorly investigated.

Here, we present our data about the identification and the detailed structural characterization of the capsular polysaccharide from both the cells and EMVs from the psychrotolerant bacterium *Shewanella vesiculosa* HM13 by NMR spectroscopy, chemical and physicochemical analyses. Our results suggest that through its adhesive properties the polymer could be involved in biofilm formation process.

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Plastic degradation potential of extremotolerant fungi screened by a novel method

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Plastic polymers are widely used because they are cheap to produce, versatile, and durable. However, their durability also causes them to accumulate in the environment. In nature, plastics gradually break down into smaller fragments and particles, known as microplastics, which can accumulate in the food chain, although the impact on human health is still unclear. Because of the major environmental and health impacts of the growing amount of discarded plastics, it is imperative that we find new ways of plastic waste management. One important but under-researched method is the biodegradation of plastics by microorganisms.

The goal of our project is to screen fungi for previously unidentified potential for plastics degradation. We are testing extremotolerant strains from our fungal collection, originating from various extreme habitats, such as those contaminated with long-chain or aromatic hydrocarbons, very cold, and/or hypersaline. We have developed a novel method to determine the plastic degradation by using gas chromatography to measure the respiration of fungi growing in the presence of plastics as the sole carbon source. This is followed by the inspection of plastic surfaces using electronic microscopy and RAMAN spectrometry. Finally, we are attempting to identify the mechanisms of plastic degradation by sequencing the genomes and transcriptomes of the best performing strains.

The most promising potential degraders of plastics have been isolated from environments contaminated by long-chain or aromatic hydrocarbons. We identified several strains of different species that increased their metabolism in the presence of various plastic polymers tested. The attachment of these fungi to the plastics was confirmed by electron microscopy. RAMAN Spectrometry showed that plastics incubated in the presence of certain fungi contained fewer ester bonds and changes in the signals of benzene rings.

Our study not only advances efforts to develop methods for microbial degradation of plastic polymers, but also contributes to the much-needed standardization of screening methods procedures in the search for the best microbial degraders.

Bacterioplankton community structure crossing the Antarctic Circumpolar Current Fronts

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The Southern Ocean is home to the world's strongest and deepest currents that control the circulation of seawater across the entire world ocean. It connects the three main ocean basins (Atlantic, Pacific and Indian) and creates a global circulation system that is largely driven by the Antarctic Circumpolar Current (ACC). The ACC flows from west to east around Antarctica and generates an overturning circulation by fostering deep-cold water upwelling and formation of new water masses. This action affects the Earth's heat balance, the distribution of nutrients in the oceans and the global distribution of carbon as well as the cycle of marine food chains. The ACC is characterized by several water mass boundaries or fronts (1), known as Sub-Tropical Front (STF), Sub-Antarctic Front (SAF), Polar front (PF), South Antarctic Circumpolar Current Front (SACCF) featured by different temperature, salinity and nutrient concentrations. The above mentioned fronts are poorly characterized from the microbiological point of view. Here, we will present the Bacterioplankton community structure crossing the Antarctic Circumpolar Current Fronts through the 16S rRNA sequence analysis obtained from surface water samples collected in the New Zealand-Ross Sea Transect during the XXXII Oceanographic cruise helded in 2017. Our results integrate microbial diversity changes with environmental conditions along the transect representing an important baseline for future studies on the response of epipelagic microbial communities to the climate changes.

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Antarctic terrestrial microbiomes in the age of climate change: resilience, sustainability and sensitivity

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The Antarctic continent, despite it's extreme geographical isolation, is highly susceptible to the effects of climate change, particularly global warming. The effects of regional climate warming on continental and maritime Antarctica are both physical and biological. Physical loss of coastal terrestrial ice mass by melting will expose new land surfaces for colonization, will contribute to surface water availability and to atmospheric humidity, cloud formation and precipitation processes.

All of these predicted effects will potentially impact on Antarctic soil microbiomes and surface 'vegetation' (mosses, lichens and algal and cyanobacterial mats). In the absence any plant vegetation in the ice-free areas of continental Antarctica, soil microbiomes play a critical role as the sole providers of ecosystem services, particularly C and N turnover. These microbial populations are also highly adapted to their 'extreme' environment: including psychrophily, freeze-thaw resistance, xerophily and resistance to oxidative stress.

The extent to which the extant biodiversity of Antarctic soils is impacted by the effects of regional climate change depends on the structural and functional **resilience** of these communities. Here we review the current state of knowledge of the resilience and sustainability of Antarctic soil microbiomes in the context of microbiome structure and ecosystem servicing.

Capnophilic Lactic Fermentation (CLF) pathway in the anaerobic hyperthermophilic bacteria of the family Thermotogaceae: occurrence and regulation

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Capnophilic lactic fermentation (CLF) is a novel anaplerotic pathway recently identified and patented in the anaerobic hyperthermophilic bacterium *Thermotoga neapolitana*. [1, 2] The CO_2-activated mechanism enables a non-competitive synthesis of hydrogen and L-lactic acid at high yields from sugar-based waste and renewable feedstocks, which makes it economically attractive for development of sustainable biotechnological processes. [3] The fermentation is dependent on a Janus pathway, which includes a catabolic branch leading to acetyl-CoA from sugars by glycolysis as well as an anabolic branch converting Acetyl-CoA and CO_2 to pyruvate by PFOR (pyruvate:ferredoxin oxidoreductase) and lactate by lactate dehydrogenase (LDH). Heterologous expression of AcetylCoA synthetase coding gene from *Thermus thermophilus* boosted acetate uptake and reductive carboxylation of acetyl coenzyme of CLF pathway in *T. neapolitana.* [4] Here I will present the molecular evidence suggesting that combination of NADH-dependent reduced ferredoxin:NADP oxidoreductase (NFN) and ferredoxin:NAD oxidoreductase (RNF) can generate a cyclic process for the supply of reduced ferredoxin and NADH that support concomitant synthesis of lactic acid and hydrogen.[5] Involvement of ATPases as essential components for managing H^+/Na^+ gradients to support CLF-phenotype will be also discussed. [6]

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A novel phylum with multiple metabolisms along the halocline of deep anoxic brine pools in the red sea

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The Red Sea hosts the largest number of chemically and physically diverse deep anoxic brine pools known so far on Earth. Laying at the bottom of the sea after originating from the dissolution of ancient evaporites, sulfidic as well non sulfidic brine pools with variable thermal profiles host a dense and stratified microbial community at the transition zones between water bodies with different salinities and densities. Variable geochemical conditions in the different brine pools have selected over time unique microorganisms that specifically occupy few tens of centimeters-thick layers of water along the chemocline/pycnocline, according to the occurring redox couples that are suitable to feed their specific metabolisms[1,2]. Contrary to most of such novel microbial taxa that have been found to inhabit only some of the different geochemical layers of the chemoclines, we have discovered a new phylum found in the non-sulfidic Suakin Deep[3] which is equipped with multiple types of metabolisms that enable to adapt to the changing conditions along the chemocline and colonize its entire vertical range. Here, the metabolic features of such novel phylum are presented together with its vertical distribution and abundance along the chemoclines of two different non-sulfidic yet physically and geochemically diverse brine pools in the Red Sea.

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Using *Thermus thermophilus* as a Whole-Cell Factory: Production of extremolytes

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Extremolytes are fundamental molecules in the physiology of extremophiles protecting cells against temperature, osmolarity and other stresses. Cyclic 2,3-diphosphoglycerate (cDPG), originally isolated from the thermophilic archaeon *Methanothermus fervidus*, naturally protects cellular proteins under extreme conditions. The biosynthetic pathway for cDPG production has been introduced into the thermophilic bacterium *Thermus thermophilus*. The two enzymes in this synthetic pathway, 2-phosphoglycerate kinase (2PGK) and cyclic diphosphoglycerate synthetase (cDPGS), were incorporated into a newly designed modular BioBricks vector. The expression of this two-enzyme cascade resulted in the whole-cell production of cDPG [1]. *In vivo* production of cDPG was confirmed by mass spectrometry to a concentration up to 650 µM. This study demonstrates the feasibility of using this well studied thermophilic bacterium as a host in a whole-cell factory approach to produce cDPG. This raises the potential for commercialisation of cDPG for cosmetic and healthcare applications and sets the ground for the application of *Thermus thermophilus* as an alternative host for other high value small organic molecules of industrial interest. Furthermore, high resolution crystal structures have been determined [2] for the native cDPGS and its complex with 2,3-diphosphoglycerate and ADP Mg^{2+}. Structural comparison identified a core domain conserved among ATP dependent enzymes but a large portion of the structure including a 127 aa N-terminal domain is unique and different from any other know protein structure. Comparison of the two structures obtained has given insight into how the enzyme changes in conformation upon substrate binding.

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The Complete Genomic Structure of the Eukaryotic Extremophile *Galdieria sulphuraria*

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Extremophiles, while typically bacteria and archaea, are also found in the eukaryotic domain of life. The eukaryote *Galdieria sulphuraria* is a thermoacidophilic red alga belonging to the class *Cyanidiophyceae*, an especially unique class as it comprises the basal clade of eukaryotic extremophiles [1]. *Galdieria* species can grow both photosynthetically and heterotrophically on a variety of carbon sources, thriving down to pH 0 and temperatures up to 56 ˚C, while tolerating high levels of reactive oxygen species and high levels of heavy metals [2]. Here we report whole-genome sequencing of six *G. sulphuraria* strains. These experiments have provided useful functional annotations and have uncovered the complete structure of three nuclear genomes. We detail the polished nuclear genome assemblies of three *G. sulphuraria* strains, uncovering a compact genome (13.1 Mb – 16.0 Mb), with 70-72 nuclear chromosomes, dependent on the strain. Preliminary comparative analyses of the macro synteny revealed that these genomes retain some structural features, even though they are estimated to have been isolated from each other for more than 10 million years, but that duplications and rearrangements have taken place. This, along with the large number of nuclear chromosomes compared to the genome size, reveals a mechanism of intrinsic adaptability in this eukaryotic extremophile, uncovering how *G. sulphuraria* can thrive in a rapidly changing extreme environment.

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Proton stress resistance, the limits of a moderate acidophilic sulfate reducing bacterium: *Acididesulfobacillus acetoxydans*

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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 precipitate metals, a valuable trait for bioremediation and biomining processes. Therefore, aSRB have been an isolation target for a long time. Initial trials resulted in isolation of neutrophiles and acidotolerant SRB which could not grow at pH lower than 3.8-4.0. This raised the question whether aSRB really exist suggesting 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. 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.

Metagenomic Microbial Community Analysis of Hydrogen Producer Thermophilic Strains of Local Hot Springs with Nanopore Sequencing

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Hydrothermal environments host a variety of unique microorganisms living under extreme conditions including high temperatures, lack of oxygen and limited nutrients. Identification of hot spring extremophiles through metagenomics is vital for the understanding of their beneficial use for different areas. Carboxydotrophic hydrogenogenic (CH) microorganisms are a class of thermophiles that have a unique pathway for hydrogen production called the Water-Gas Shift (WGS) reaction. CH microorganisms utilize carbon monoxide (CO) into hydrogen (H_2). This provides a clean energy source H_2 by reducing toxic CO levels and plays a vital role in the microbial ecosystem. Hydrogen being considered as clean fuel and a promising alternative to commercial fossil fuels. This study aims to identify thermophilic isolates and to determine their biohydrogen production capacity.

Experimental

Isolation of thermophilic isolates was performed from 5 different local hot springs in Izmir. The hot springs include thermophilic (42-77.3°C) environments and pH is in a range of 6.42 to 7.88. Isolates were cultured in anaerobic media with 100% CO under batch, thermophilic (60°C) conditions. The enriched cultures were screened for biohydrogen production using Gas Chromatography-Thermal Conductivity Detector(GC-TCD). A metagenomic analysis was performed with 16S rRNA amplification products using a MinION Sequencer from Oxford Nanopore Technologies™.

Results and Discussion

Metagenomic analysis revealed that Novosphingobium species dominated all hot spring microbial communities (61%). Hot spring 1 (HS1) showed five times more hydrogen yield and 16S analysis verified that microbial consortia included CH organisms with taxonomically assigned genus of 24% Caloramator and 18% Moorella species. The lowest production was seen in the Hot Spring 4 (HS4) with a microbial profile consisting mainly of Novosphingobium(75%).

Conclusions

Overall, our results demonstrated a significant metagenomic characterization of the microbial communities from local hot springs assisted in understanding the microbial dynamics to verify and improve biohydrogen yields.

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Toolbox for molecular acclimatization of haloarchaea within salt crystal

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Halite salt crystals (NaCl) can host with halophilic microorganisms trapped within the fluid inclusions generated during evaporation. These halophilic microorganisms are hypothesized to be preserved and even remain viable over extended time periods, although the exact duration of viability is still unclear [1]. However, the molecular mechanisms involved in their acclimatization to conditions within halite fluid inclusions are still poorly understood. Important questions remain unanswered regarding cell viability, activity, metabolism, and structural modifications after their entrapment.

Saturating salt conditions, changes induced in halophiles immediately during/after rehydration of halite, and the difficulties associated with the isolation of biomolecules from halite fluid inclusions (without contamination by surface-bound cells or biomolecules) all pose significant technical challenges to the development of reproducible analytical approaches. Previous studies have focused mainly on isolation of DNA, with different methodologies utilized to extract and analyze DNA from surface-cleaned crystals [2,3]. However, extraction of other biomolecules such as proteins and RNA have not been reported, despite extensive proteomic and transcriptomic analyses available for liquid cultures of the model haloarchaeon *Halobacterium salinarum* str. NRC-1 [4,5].

To overcome these challenges, we have developed a new toolbox for biomolecule extraction and analysis by "-omics" approaches directly from surface-cleaned laboratory-made halite with *H. salinarum* cells trapped inside fluid inclusions [6]. This new toolbox will enable new insights into molecular acclimatization of halophiles in halite under a range of conditions (lab-grown or natural samples).

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Methylotrophy in the deep: metagenomics and physiology of microbial enrichments from Black Sea sediments

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Methylated compounds, such as methanol or trimethylamine (TMA) are common compounds in anoxic marine sediments as end products of fermentation of organic osmolytes. These compounds can be used by different types of anaerobic microorganisms, such as methanogens or sulfate reducing microorganisms (SRM). As such, these microbial metabolisms have the potential to thrive on methylated compounds in deep-sea sediments, with impact in the global carbon and nitrogen cycles. However, very little is known about the physiology and specific interactions of individual species, from different metabolic groups growing on methylated substrates. The Black Sea is an excellent proxy to study this, as it is the largest permanent anoxic and sulfidic basin in the world and harbours high microbial diversity. To this end, we sampled the top 30 centimeters of Black Sea sediments at 2000 meters below sea level. These samples were used for starting-up microbial enrichments, which were monitored by 16S rRNA amplicon sequencing and metagenomic analysis through Illumina MiSeq sequencing. Methanol was selected as carbon and energy source and two series of enrichments were developed using specific inhibitors to enrich for SRM and methanogens. Initial community analysis of the sediment revealed a broad community with potential of utilizing methylated compounds. After 24 months of enrichment, stable enrichment cultures were reached, fuly depleting methanol and producing sulfide and methane in SRM and methanogenic enrichments, respectively. Metagenomic sequencing revealed that the SRM enrichments were dominated by the genus *Desulfosporosinus*, with additional presence of microorganisms from the families *Marinifiliaceae, Halanaerobiales* and *Anaerolineaceae*. Methanogenic enrichments were dominated by the genus *Methanococcoides,* with additional presence of *Marinifiliaceae* and *Izemoplasmataceae*. These results will yield a deeper understanding of the global carbon and nitrogen cycles in piezophilic, sulfidic and anoxic environments and their influence on their global cycles.

Elemental Sulfur Disproportionation: Overcoming Energetic Barriers for Biotechnological Application

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Sulfur disproportionation is an ecologically and technologically relevant but poorly understood part of the sulfur cycle[1]. The process is endergonic at standard physiological conditions, but thermodynamically favorable under specific circumstances, in which sulfide is scavenged by iron/manganese oxides[2]. Redox conditions play a crucial role in the growth and activity of the cells, allowing only a very short range of opportunities for the process. Addition of metal oxides and precipitation as metal sulfides, however, impose some obstacles to proper investigation of the biomass growth and viability. Alternative strategies to guarantee the removal of sulfide from the system without compromising growth and activity of the cells is of importance to foster studies on the field. This research reports a comparative assessment on growth and activity of the sulfur-disproportionating bacterium *Desulfocapsa sulfexigens* by applying multiple strategies of sulfide removal from triplicate batches: 1) Cultures were incubated with 30mM FeOOH as sulfide scavenger. 2) Cultures were constantly sparged with N_2/CO_2 (10 to 20mLmin^{-1}, and 1mm pore size) and stirred (25 to 200rpm). 3) Cultures were continuously sparged (0.5µm pore-size) through to the liquid phase and N_2/CO_2 was flushed through it at 5mLmin-1 flow. 4) Cultures were connected to spargers (0.5µm pore-size) and sulfide was stripped out by flushing in N_2/CO_2 at 5mLmin-1 flow in pulses, when stationary phase was achieved. Sulfate, sulfide, 16S rRNA copy genes and number of cells were monitored. Zinc precipitation was analyzed by conversion of sulfide measurement. All conditions showed growth and activity dependent on a small range of redox to kickstart the process. This study shows the suitability of S^0 disproportionation for metal recovery, allowing the stripping of sulfide and surpassing activity (≥ 30 mM sulfide) and growth of the conventional strategies applied (± 20 mM sulfide), in a clean culture. Transcriptome analyses will be further performed to shed light on the pathways involved in disproportionation of zero-valent sulfur, helping to develop tools for optimization of their performance targeting biotechnological applications.

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Exploring the carboxydotrophic potential of microbial communities in hydrothermal areas

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Carbon monoxide (CO) is a potential carbon and energy source for anaerobic microbes in hydrothermal environments. Thermophilic carboxydotrophs (i.e. microorganisms that can use CO) are of biotechnological interest, since they can be employed in industrial processes, such as syngas fermentation, for the production of chemicals and fuels. To date, a small number of thermophilic carboxydotrophs have been isolated from hydrothermal areas [1,2,3] and the vast majority of these environments remains unexplored for this type of metabolism. In the present study, we explored samples from different hydrothermal sites in the island São Miguel (Azores, Portugal) for their carboxydotrophic potential. We succeeded in obtaining 19 thermophilic enrichment cultures that are consistently consuming CO and producing various compounds including H_2, methane, acetate, and propionate. Microbial composition varies in the different enrichment cultures. Most of them are dominated by microbes affiliated with carboxydotrophs, such as *Moorella* and *Methanothermobacter* closely related species. However, some of the enrichments show high abundance of bacteria closely related to *Thermoanaeromonas* and *Tepidanaerobacter* species, which have never been reported for CO oxidation. The results from this study contribute to our fundamental understanding of the metabolic potential of the microbes inhabiting hydrothermal environments and are guiding the isolation of some novel thermophilic carboxydotrophs with potential for industrial applications.

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Proterozoic park. Microbial communities of subsurface mineral waters mirror the earth's ancient biosphere

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Continental subsurface ecosystems are composed of rocks formed over the entire period of the Earth's geological history. Various magmatic, metamorphic, and sedimentary rock combinations formed the environments holding the records of the highest registered temperature, pressure, pH, and salinity. The inhabitants of these hostile biotopes are estimated to constitute >10% of our planet's total biomass. In a limited set of sampling sources for their investigation, deep subsurface mineral water aquifers remain essentially unacknowledged. Within an interdisciplinary study, we have monitored the chemical composition, phylogenetic and metabolic diversity of microbial communities at six different aquifers of Yessentukskoye mineral water deposit (Pre-Caucasus region) over the 3-year period. Thermal carbonaceous mineral waters from the Upper Cretaceous aquifer revealed the composition close to that presumed for the Proterozoic ocean. 16S rRNA profiles of several microbial communities, sampled from this same aquifer, significantly changed down its depth but were stable for each site over the sampling period. Deep phylogenetic lineages of uncultured microorganisms, such as Archaea of *Hadarchaeales* phylum or *Actinobacteria* of novel classes and orders, were highly represented in these communities. Beta diversity calculations outlined a group of prokaryotic taxa peculiar for the deep aquifer and not occurring in the upper zone of active hydrodynamic water exchange. Metagenomics-based optimal growth temperature calculations indicated these taxa to be thermophiles. MAGs analysis indicated methanogenic or acetogenic autotrophs to be the most represented metabolic groups in Proterozoic-like thermal waters, with Fe(III)-reducers being the second abundant group. An Fe(III)-reducing actinobacterium of OPB41 lineage was isolated into a pure culture, as well as several methanogenic Archaea. The biodiversity we have traced in our survey could reflect the composition of early Earth's biosphere, as it experienced low evolutionary pressure due to high stability of physico-chemical conditions in continental water-bearing rocks over the geological time scale.

The work was supported by the RSF grant # 21-14-00333.

Geosphere and Biosphere coevolution: the role of trace metals availability in the evolution of biogeochemistry

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Earth's geosphere and biosphere have coevolved over time, influencing each other's stability and keeping our planet habitable over the last ~4 billion years[1]. Biogeochemical cycles play a key role in controlling this interaction, connecting long-term geological cycles and the much faster evolution of the Earth's outer biologically dominated envelopes[2]. A small set of microbial-encoded proteins containing redox-sensitive transition metals as their core catalytic center carry out the majority of the key biogeochemical reactions. Metals such as Fe, Co, Ni, Zn, Mo, W, V, and Cu are used in these proteins to access diverse redox couples as a function of the changing planetary availability of these elements over time[3]. Despite the importance of this process, the relationship between metal availability and metabolism evolution and diversity has not been investigated in detail[4]. Here, we will present recent data from field and laboratory experiments elucidating the impact of transition metal availability on microbial functional diversity, and its implications for the emergence and evolution of life on Earth, while discussing the implications of this findings for the search for life in the Universe.

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Archaeal dominance in low-temperate acidic environment

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Archaea in comparison to bacteria considered to be significantly underrepresented in low-temperature acidic environments. The current acidophilic microbiology recognises temperature as a main factor influencing archaeal representation in microbiomes, together with pH, and other physico chemical parameters. The microbiome of acidic setting Parys Mountain (UK) characterised by constant moderate temperatures (8-18 ºC), low pH (1.7) and high quantities of metals and metalloids was objected to check the generality of this presumption[1, 2]. Metagenomic and SSU rRNA amplicon sequencing of DNA from sediment surface and layers underlying acidic stream revealed numerically dominant archaeal signatures, affiliated to *Thermoplasmata* and *Ca*. Micrarchaeota. All archaea accounting maximally in surface sediment microniche (67% from total reads). Correlation between the presence of *Thermoplasmatales* and sediment Fe, As, Cr and Mn contents was noted. One group of *Thermoplasmatales*, E plasma cluster, represented by uncultured organisms was found as a major group (58%), implying on massive accumulations and functional importance of these archaea in the environment[1, 2]. Potential mechanisms contributing to ability to thrive at lower temperature for some archaeal species were exposed [3]. Alongside with E plasma other archaea of the order *Thermoplasmatales* and "Terrestrial Miscellaneous Euryarchaeal Group" (TMEG) detected in minor proportions with yet unclear functional roles in the ecosystem were revealed. Our global analysis of TMEG representation in metadata showed that sequences from acidic sites forming a monophyletic group on the level of order within *Thermoplasmata*. The main factors favouring Parys Mt archaeal numbers were concluded pH and conductivity but not temperature[1, 2].

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The response of a thermophilic bacterium to near-zero growth. A transcriptomic analysis

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Microbes in the environment are believed to be exposed to feast-famine cycles with frequent and long periods when they persist strict nutrient-limitation conditions[1, 2]. Thus, microorganisms in nature must adapt to thrive through adverse conditions showing during these periods extremely reduced, near-zero, growth. To better understand the behavior of microorganisms under those conditions, we cultured a soil thermophilic isolate, *Parageobacillus thermoglucosidasius* strain 23.6, in a retentostat system [3] in order to achieve near-zero growth rates with doubling times above 150 days. Using transcriptomic analysis, by comparison of global gene expresion in cells at optimum growth rate (18 min) with cells at near-zero growth, we attempt to elucidate some of the mechanisms of response of *P. thermoglucosidasius* 23.6 to severe nutrient-limitation. Results showed that these cells switch their main metabolic pathways activating secondary metabolite production and the use of alternative nutrients besides the inhibition of cellular division processes. This study contributes to comprehend how bacteria, specifically *P. thermoglucosidasius* 23.6, thrive through nutrient scarcity and so persist in the environment during periods of nutrient depletion.

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Recruiting deep subsurface microbiomes to sustainably sequester carbon

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Elevated atmospheric carbon dioxide (CO_2) levels prevent re-radiation of long wavelength radiation, resulting in a 'greenhouse effect' that is affecting global climate. The most sustainable and cost-effective process of capturing CO_2 from the atmosphere is photosynthesis. Biomass consists mostly of cellulose, hemicellulose, and lignin, these macromolecular carbon structures are robust and difficult to degrade. Specialized microorganisms can degrade and eventually re-release the carbon back into the atmosphere over time, this appears as a seasonal atmospheric flux of CO_2. As the concentration of CO_2 steadily increases due to the burning of fossiliferous carbon-based materials, it becomes clear that encouraging photosynthesizing organisms to fix and store carbon is a logical strategy to address global climate change, however, deciduous plant life does not permanently sequester carbon.

The recalcitrant nature of cellulosic biomass is a major impediment, especially when carbon is to be sequestered deep underground. Oxidative hydrothermal dissolution (OHD) can break down waste biomass into low molecular weight water-soluble products. Carbon in this form is non-toxic, easily transportable, and amendable to a wide variety of microorganisms. The deep subsurface is home to many uncharacterized microbial communities. The effects and biochemical fate of complex soluble carbon streams from OHD on subsurface microbial communities is currently unknown. Next generation sequencing and geochemical analysis have revealed which subsurface microorganisms are recruited that can metabolize OHD liquid carbon. Injection of bio-derived, water-soluble organic materials into the deep subsurface provides a plausible route for long term sequestration of carbon, effectively creating a carbon negative atmosphere-to-geosphere counter-flux capable of capturing and removing carbon from the earth's atmosphere.

Polyphosphate metabolism in the thermoacidophilic Crenarchaeon *Sulfolobus acidocaldarius*

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Inorganic Polyphosphatepolyphosphate, a linear polymer of orthophosphate residues linked by phosphoanhydride bonds, occurs in all three domains of life and plays a diverse and prominent role in metabolism and cellular regulation[1](1). While the polyphosphate metabolism and its physiological significance have been well studied in bacteria[2] (2)and eukaryotes including human[3](3), there are only few studies in archaea available so far. Until now, different types of polyphosphate kinases have been reported but despite intense investigation, the nature of the crenarchaeal polyphosphate kinase is still unknown[4](4). In Crenarchaeota including members of *Sulfolobales*, the presence of polyphosphate and degradation via exopolyphosphatase has been described and there is some evidence for a functional role in metal ion chelation, biofilm formation and motility[5](5). Here we used the crenarchaeal model organism *Sulfolobus acidocaldarius* to study the enzymes involved in polyphosphate metabolism. The recombinant exopolyphosphatase exhibited high specific activity with medium chain polyphosphates ($PolyP_{45}polyP_{45}$, 857 U/mg protein) although highest activity was observed with long chain polyphosphates ($PolyP_{700}polyP_{700}$). In addition, we identified a putative polyphosphate kinase by comparative bioinformatic analysis. The enzyme was expressed, purified and characterized using enzymatic assays as well as ^{31}P-NMR spectroscopy confirming the predicted polyphosphate kinase activity. The current insights in polyphosphate metabolism and function in *S. acidocaldarius* and novel phylogenetic implications will be discussed.

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Cs^+ tolerance mechanisms of high-concentration Cs^+ tolerance bacterium alkaliphilic *Microbacterium* sp. TS-1

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Cesium (Cs) is a kind of alkali metal, isotopes (^{134}Cs and ^{137}Cs) are radionuclides, and ^{137}Cs is regarded as a major cause of radioactive contamination [1]. In 2013, we isolated alkaliphilic *Microbacterium* sp. TS-1 (TS-1) from jumping spider ground extract and reported its genomic sequence [2]. The optimum growth pH of this bacterium is 9.0, and it can grow even in a medium containing 1.2 M CsCl. Therefore, it is expected that strain TS-1 has a unique Cs^+ resistance mechanism. In this study, to clarify the Cs^+ resistance mechanism of strain TS-1, Cs^+ sensitive mutants by chemical mutagenesis treatment and their spontaneous revertant strains from Cs^+ sensitive mutants. The mutation sites of these strains were clarified by whole-genome sequence analysis, and we identified the genes involved in Cs^+ resistance (*MTS1_00475* and *MTS1_03028*). *MTS1_00475* is encoded a Cs^+/H^+ antiporter [3]. The apparent K_m value for Cs^+ at pH 8.0 was 250 mM. On the other hand, *MTS1_03028* is an encoded Mg^{2+} transporter (*mgt*). Adding $MgCl_2$ in the medium of the Cs^+ sensitive *MTS1_03028* defective mutant, the mutant restored the Cs^+ resistance to the same level as the wild type. This suggests that Mg^{2+} uptake plays an essential role in Cs^+ resistance in TS-1. Our results indicated that the Cs^+ resistance mechanism of TS-1 is inferred as follows. When the strain TS-1 is exposed to 100-200 mM Cs^+, TS-1 gains Cs^+ resistance by increasing the intracellular Mg^{2+} concentration. However, when exposed to Cs^+ exceeding 200 mM, the Cs^+/H^+ antiporter encoded by *MTS1-00475* extrudes the high concentration of Cs^+ taken up from the cell and always lowers the intracellular Cs^+ concentration. As a result, tstrain TS-1 can grow even in a high Cs^+ concentration environment.

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From the surface to the bottom of the ocean: a novel instrument to investigate the effect of hydrostatic pressure on microbially influenced corrosion

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Microbially Influenced Corrosion (MIC) is the process that occurs when microorganisms, including many Bacteria and Archaea, either accelerate or slow down the corrosion processes driven by the exposure of the metal to the environmental conditions.

Despite the fact that MIC has been studied for over a century, certain elements of its mechanisms remain controversial and rarely studied in extreme environments. For example, the ocean floors have been increasingly exploited for deployment of communication cables, oil and gas pipelines, scientific studies (e.g. simulation of extra-terrestrial ecosystems), and mining of precious metals. Yet, very little is known about the corrosion dynamics to the structures and instruments once they are submerged. Other chemical and physical parameters can be easily investigated or even analysed using models, but studying the biotic component requires expensive and time-consuming *in-situ* testing or laboratory analysis.

Here we describe a continuous-flow bioreactor to study deep-sea MIC in the lab, that can simulate high hydrostatic pressure (HHP) up to 60 MPa while flushing out metabolites and by-products. This procedure ensures longer incubations and keeps the cultures in exponential growth phase. It can also be operated anoxically for the incubation of obligate anaerobes while performing real-time electrochemical analyses.

Testing the bioreactor on pure cultures of sulphate reducing bacteria (SRB), revealed that HHP can raise or decrease corrosion rate depending on the SRB species and the nutrient regimes.

Using the bioreactor with communities enriched from deep-sea sediments can also assist in gaining a true understanding of the processes driving MIC in this harsh environment and provide strategies for mitigation.

Geochemical forcing causes extensive functional diversity in an abundant (hyper)thermophilic archaeon in Yellowstone National Park

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Yellowstone National Park, USA (YNP) contains an enormous diversity of geothermal features (>14,000) and provides a remarkable natural laboratory for studying linkages between microbial function and geochemistry in extreme environments. The distribution, diversity, metabolic capability, and activity of abundant, autochthonous YNP *Pyrobaculum* populations were analyzed using metagenomes, metatranscriptomes, single amplified genomes (SAGs), existing isolate genomes, and long-term geochemical datasets obtained from high-temperature habitats (65 – 90 °C, pH 6 – 9). Highly related *Pyrobaculum* populations (>90% ANI) obtained across sampling locations shared many core genes with *P. yellowstonensis* WP30, the only isolate cultivated from YNP. However, metabolic reconstruction revealed unexpected differences in potential respiratory pathways specifically related to the predominant geochemistry of different springs, including O_2 (types of reductases), N (denitrification), As (oxidases-reductases), and various S species (DMSO-molybdopterins). Genome sequence of individual *Pyrobaculum* cells (SAGs) further supported a notable level of genomic and functional heterogeneity within the same spring. *Pyrobaculum* transcripts were also compared between a sulfidic hot spring and a geochemically similar oxic spring and showed *Pyrobaculum* populations were very active members of both communities and a large proportion of their transcripts mapped to genes related to concentrations of O_2, sulfide, and arsenite. The predominant geochemistry of different hot springs selects for various metabolic combinations in closely related YNP *Pyrobaculum* populations, thus this 'geochemical forcing' of genomic properties reveals an extensive diversity of possible physiologies in native *Pyrobaculum* and provides an important mechanism for resilience and adaptation in response to changing environmental conditions.

Transcriptome Analysis of Halotolerant Staphylococcus saprophyticus Isolated from Korean Fermented Shrimp

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The high salinity in salted fermentation foods is known to contribute to its flavor and inhibit the growth of unfavorable microorganisms. Saeu-jeotgal, a Korean fermented shrimp food, generally has 35-40% salt concentration which is known to higher than other type of jeotgals. Staphylococcus saprophyticus was isolated from the saeu-jeotgal and discovered to be capable of growth even after treatment with 20% NaCl. To elucidate the salt tolerant mechanism of the organism, transcriptome analysis was conducted using RNA sequencing against 0%, 10%, and 20% NaCl. At the time point after 6 h from salt treatment, 831, 1314, and 1028 differentially expressed genes (DEGs) were identified in the 0% vs. 10%, 0% vs. 20%, and 10% vs. 20% NaCl comparisons, respectively. Clusters of Orthologous Groups analysis revealed that the DEGs were involved in amino acid transport and metabolism, transcription, and inorganic ion transporter and metabolism. Functional enrichment analysis showed that the expression of genes encoding mechanosensitive ion channels, sodium/proton antiporters, and betaine/carnitine/choline transporter family proteins were downregulated, whereas the expression of genes encoding universal stress proteins and enzymes for glutamate, glycine, and alanine synthesis were upregulated. Judging from the overall analysis of the salt tolerance mechanism, glutamate was considered as a key intermediate molecule in this strain. Therefore, these findings suggest that S. saprophyticus isolated from saeu-jeotgal utilizes compatible solute strategies with glutamate for halotolerance.

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Poly-extreme environments: promising Sources of novel enzymes for sustainability

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Enzymatic reactions are safer, faster, less hazardous, and generate less waste, thus following the twelve rules of green chemistry[1]. Enzymes originating from harsh environments offer exceptional stability under extreme conditions and are therefore one of the main pillars of sustainability[2]. However, understanding and harnessing of life under extreme conditions are challenging due to the difficulties of *in situ* observation and the lack of cultivatable organisms (> 99% can't be cultivated)[1,3]. We used culture-dependent and culture-independent methods to comprehensively assess the structure and function of polyextremophilic enzymes from Deep Lake of Antarctica (−18 °C to +11.5 °C and 21–28%, w/v salt content) and Red Sea brine pools (23 °C to 68 °C and 26–33%, w/v salt content, high metal content)[4-7]. The enzymes were expressed in a halophilic expression system, purified, structurally, and functionally characterized. Indeed, purified enzymes showed activity under extreme temperatures, high salt concentrations, and organic solvents. Our crystal structures of extremozymes combined with biochemical analyses provide insights into how enzymes adapt to these extreme conditions. The findings will facilitate the bioengineering of enzymes with valuable properties for green, sustainable, biobased economy, and biotechnology.

Keywords: Extremophiles; extremozyme; halophiles; polyextremophiles; thermophiles, sustainability

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Cloning, Biochemical characterization and comparison of active site mutations at subsite +2 of *Anoxybacillus ayderensis* A9 Beta-glucosidse for hydrolysis of *p*NPG and polydatin

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This study reports a novel BglA9 gene of 1345 bp encoding β-glucosidase from *Anoxybacillus ayderensis* A9*,* which was amplified and expressed in *E. coli* BL21 (DE3): pLysS cells, purified with Ni-NTA column having molecular weight of 52.6 kDa and was used in the bioconversion of polydatin to resveratrol. β-glucosidase from *Anoxybacillus ayderensis* A9 (BglA9) is a potent enzyme for enzymatic hydrolysis of polydatin to resveratrol. Based on structural and bioinformatics analysis an area near +2 subsite of the active site pocket of BglA9 was selected and single point mutations were introduced with the aim to enhance the substrate specificity of the enzyme towards *p*NPG and polydatin. The changes introduced in the active site residues were L221S, N222S and G226Q. All the mutants were expressed in *E. coli* BL21 (DE3) cells and purified with Ni-NTA column chromatography. All the mutants retained their thermal and pH stability and the best mutant in terms of catalytic efficiency for *p*NPG and polydatin was N222S. The docking analysis supported the results and by comparing binding energies; the mutant N222S showed the best docked complex. This investigation suggests that +2 subsite of BglA9 is an interesting area to be mutated for enhanced catalytic efficiency for *p*NPG and polydatin. The deglycosylated derivate showed enhanced antioxidant potential as compared to glycoside measured by DPPH assay.

Keywords: β-glucosidase, Polydatin, *Anoxybacillus ayderensis* A9, Active site mutations, Rational designing**.**

Preparing for Real-time Monitoring of Halophilic Archaea Exposed to Space Radiation Outside the International Space Station on Exocube

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High-salt environments provide a potentially favorable geobiological context for protecting extant life and preserving biosignatures of past life, particularly from halophilic microorganisms trapped within brine inclusions. Extensive study of the model haloarchaeon *Halobacterium salinarum* have shown that this extreme halophile is highly resistant to desiccation, high vacuum, UV-C, and gamma irradiation[1,2], in part to the anti-oxidant properties of salts, both intracellular[3] and extracellular. High salt environments are not only found on Earth. Evidence for past and present high salinity conditions are found everywhere from Enceladus and Europa to Mars. Organic molecules (of a non-biological origin) have been preserved within the brine inclusions of extraterrestrial halite crystals found within meteorites. Halophilic archaea have been extensively used as models for astrobiology (ground-based and space-based) but to date only post-flight analyses have been conducted due to technical constraints with spaceflight experiments.

In the ExocubeHALO project, we will extend these studies to monitoring the real-time effects of space radiation outside the international space station on halophilic archaea cell integrity/activity and cell envelope stability as part of the ESA Exocube experiment (PI Andreas Elsaesser). Here we present our preliminary work to develop a reporter dye system and spectroscopic[4] methods compatible with spaceflight requirements and hypersaline conditions, and what this work contributes to the overall field of halophile microbiology and the preservation of halophilic biosignatures.

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Visualization and identification of metabolically active microbial populations in hot spring biofilm

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The composition and activity of select populations in microbial hot spring biofilms is strongly influenced by fluctuations in temperature and the availability of electron donors and acceptors. To gain further insights into these complex communities, we characterized the microbial communities by 16S rRNA gene amplicons sequencing, and identified active populations under different substrate amendments and incubation conditions. Bioorthogonal noncanonical amino acid tagging (BONCAT) was applied to reveal active cells by tracking the incorporation of synthetic amino acids into newly synthesized proteins, which are later detected by fluorescent staining using azide-alkyne click chemistry. Subsequently, the phylogenetic identity of translationally active cells was determined by combining BONCAT with catalyzed reporter deposition rRNA-targeted fluorescence in situ hybridization (CARD-FISH). Interestingly, the anabolic activity of *Gammaproteobacteria*, *Campylobacterota* and *Chloroflexi* was increased in all incubations regardless if organic or inorganic substrate was amended, but the highest activity was observed in glucose and acetate amended incubations.

Metabolic dependent growth behavior of *Thermococcus kodakarensis*

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Thermococcus kodakarensis is a heterotrophic hyperthermophilic archaea that grows optimally at 85°C in the presence of elemental sulfur and has a wide temperature range of growth from 60-100°C. Previous studies show that the requirement of elemental sulfur is relieved when cultured in the presence of pyruvate suggesting the metabolic flexibility of *T. kodakarensis* (Respiration of S^0 VS Fermentation) [1]. Despite this, there is no clear information about the growth behavior of *T. kodakarensis* under different substrates and different temperature conditions. Further, the energy dynamics of the system and how it varies across different temperatures are rarely assessed. The aim of this study is to monitor the growth dynamics of *T. kodakarensis* under different growth conditions and to comment on the cellular energy dynamics through ATP measurements. We hypothesize that the different substrates trigger different metabolic pathways and as a result, at a given temperature, the growth rate is altered. To address this, we culture *T. kodakarensis* at different temperatures (60, 65, 70, 75, 80, 85, 90, 95, and 100°C) and under different substrate conditions (S^0, pyruvate, colloidal S, and both S & pyruvate). The results suggest that temperature variation not only alters the growth rate but also the carrying capacity of the media. Further by culturing in different substates and measuring the intracellular ATP, we show that at a given temperature, the growth rate, the optimal temperature for growth, and the intracellular ATP are dependent on the metabolic pathways involved. Additionally, it was observed that the cell sizes remained constant under different conditions. These results support our hypothesis that the effect of temperature on metabolism causes the variation in growth rate. Our work demonstrates how a substrate, and its associated metabolic pathway can play a role in determining the optimum growth temperature of an organism. This study is of significance to exobiological research as these results will be used to understand the thermal limit of life based on the metabolism and energy dynamics of a cell.

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Deep underground microorganisms for co_2 subsurface recycling

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The dramatic increase of anthropogenic greenhouse gases concentrations in the atmosphere threatens the ecosystems equilibrium. A promising strategy consists in storing the CO_2 in deep geological formations, like deep saline aquifers. However, most of the considered methodologies aim at forming carbonates to safely store CO_2 as a waste. Oppositely, it could be used as a raw material for producing valuable resources such as methane. The transformation of part of the stored CO_2 into methane would lower the costs of capture and storage and change the paradigm of CO_2 geological storage.

Deep saline aquifers are porous environments, with temperature ranging between 40°C to 100°C and hydrostatic pressure up to 150 bar. When CO_2 is injected into these environment, several geobiochemical relations can proceed, in particular with methanogens microorganisms, which are part of the microbial population living in deep saline aquifers. Specifically, one can consider hydrogenotrophic methanogens, which can transform CO_2 and H_2 into methane, through methanogenesis: $CO_2 + 4\ H_2$ à $CH_4 + 2\ H_2O$. Using their metabolisms would allow considering deep saline aquifers as macro bioreactors for an upgrading process of CO_2 to methane, which is reusable to generate energy, through combustion, the produced CO_2 being reinjected to loop the process.

To investigate the transformation of CO_2, we have developed multiscale high-pressure transparent biocompatible approaches (microfluidics and millifluidics) allowing mimicking deep environments at lab scale. The approaches make it possible to simulate the deep ecosystems, to estimate the possibility of testing exploitation scenarios.

It was found that microbial CO_2 conversion to methane can reached high conversion rates. This is also linked with the development of biofilms inside the reactor, which was monitored using microfluidics approaches.

Therefore, the promotion of the biochemical conversion of CO_2 to methane in geological formations constitutes an advantageous long-term CCUS strategy. Deep saline aquifers may represent an interesting option for stable long-term energy generation and storage in addition to being a cost-effective strategy for CO_2 valorization at a global scale.

The Evolution of Environmental Adaptation in ATP synthases: From Extremophiles and Parasites to Mammals

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When it comes to a challenging environment, aerobic thermoalkaliphiles clearly enjoy a challenge. What is logical about their physiology is that like most thermophiles they all use sodium ions to drive substrate import. This is a prudent choice, because sodium ions are roughly 1000-fold less permeable across a biological membrane than protons. However; strangely the respiratory chain, the cells means of generating cellular ATP, is proton-coupled. This biological oddity has been the focus of several careers spanning the last 4 decades. What then compounds this matter is that these organisms also grow optimally at pH 9.5 in a virtual proton desert. The combination of these two biological pressures falls very heavily on one enzyme in particular, natures smallest molecular motor, the proton coupled F-Type ATP synthase.

In this presentation I discuss the state of understanding of life at the edge of alkaliphily and temperature. I broadly cover the energetic adaptations they have undergone to thrive under such hostile conditions. I specifically focus on the energetics of the F-type ATP synthase, nature's smallest rotary motor, discussing the intricate molecular adaptations that tune the motor to not only capture protons efficiently at alkaline pH, but also how the F-type ATP synthase has developed specific mechanical features preventing energy loss. We have explored these features now for over two decades and have expanded this to be able to compare with mammalian and parasitic systems lack these adaptations, proving function and providing ground-breaking new evidence on how energy generation with the ATP synthase adapts to pH. Perhaps unsurprisingly, many of these adaptations are in fact pH-regulated!

Functional analysis and structure prediction of DNA polymerase I from the extremophile *Deinococcus radiodurans*

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DNA polymerases are multifunction enzymes involved in recombination, replication, and DNA repair, including the highly conserved Base Excision Repair (BER). Here we focus on DNA polymerase I from the extremely radiation resistant bacterium *Deinococcus radiodurans* (DrPolI). We have characterized its functions associated with BER; DNA gap filling (GFA), DNA strand displacement (SDA) and 5´flap endonuclease (FEN) activities. Moreover, we predicted and analyzed the structure of the full-length apo-DrPolI by combining cryogenic Electron Microscopy (cryo-EM) and protein homology modeling. Based on our functional characterization, we propose that DrPolI is the main polymerase in long-patch BER, but that it also participates in short-patch BER. The structural prediction of apo-DrPolI indicates that the N-terminal domain (NTD) is mobile, as it was undetected in single particle cryo-EM analysis (6.8 Å). The flexible NTD may also explain the ability of PolI to switch between multiple activities. Our findings give insight into the functional transition modes of DrPolI during BER as well as the BER downstream mechanisms in bacteria with multiple polymerases.

Funding acknowledgements*:*
This work benefited from access to the Instruct-ERIC Centre Finland and financial support was provided by Instruct Internship Grant: APPID926 and Access Grant: PID 10934. We acknowledge the financial support of IMpaCT (imaging life from molecules to cells), this project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857203. We thank the IMpaCT team for being the bridge to contact cryo-EM partners, to access resources and workshops for knowledge transfer.

Polar microalgae: ecophysiological diversity and their potential for low-temperature biotechnology

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Microalgae represent a very successful group in various polar habitats. To withstand the extreme environment, they evolved a wide range of adaptations that help to protect them against cold, freeze-thaw cycles, desiccation, and highly variable solar radiation[1]. As result, they can grow at temperatures close to zero and produce a tremendous diversity of specific compounds, which makes them prospective candidates for sustainable low-temperature biotechnology. Here we focused on a set of strains of green algae (Chlorophyta) from our working collection that were isolated from freshwater, snow and soil samples collected during several expeditions to Svalbard (High Arctic) and James Ross Island (Antarctica). The strains from the genera *Monoraphidium*, *Bracteacoccus*, *Neocystis*, *Chodatodesmus* and others were studied using polyphasic approach that included their molecular and ecophysiological characterization. If the necessary requirements were met (namely high growth rates at low temperatures and production of valuable compounds as polyunsaturated fatty acids and carotenoids), this was followed by cultivation tests on medium to large scale. An open thin-layer photobioreactor (volume 150 L) was successfully used for biomass production under winter conditions of Central Europe[2]. Further, a closed flat panel type bioreactor (volume 20 L) equipped with automated rotation mechanism that follows the sun was newly developed. It allows to maximalise the capture of photosynthetically active radiation under conditions, when light represents the main factor limiting algal growth (winter period in temperate zone, summer in polar regions). The pilot tests must be followed by a multi-criteria evaluation considering technical, environmental, and economic aspects at industrial scale.

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Purification of heterologously-produced aldehyde: ferredoxin oxidoreductase (AOR) from *Thermoanaerobacter* sp. strain X514

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With the increasing need for alternative liquid fuels, production of alcohols from biomass and from synthesis gas (mainly H_2, CO, CO_2) becomes important[1]. Promising whole-cell biocatalysts are anaerobic microorganisms utilizing a pathway involving aldehyde:ferredoxin oxidoreductase (AOR) and alcohol dehydrogenase (ADH). In this AOR-ADH pathway AOR catalyzes the reduction of organic acids to aldehydes with reduced ferredoxin as electron donor. The aldehydes are subsequently reduced to the corresponding alcohol by an ADH. AORs are oxygen-sensitive proteins containing tungsten and they have wide substrate spectra[2,3].

Our goal is the biochemical characterization of AOR from *Thermoanaerobacter* sp. X514. *Thermoanaerobacter* spp. are thermophilic sugar fermenters and well known for their high ethanol yields; with one known exception: the acetogen *Thermoanaerobacter kivui*. Since *T. kivui* is genetically accessible[4] and has no *aor* in its genome, a His-tagged version of *aor* from *Thermoanaerobacter* sp. strain X514 was heterologously expressed in *T. kivui*. Active AOR-His was purified from *T. kivui*, as we showed by Western Blot analyses and enzyme assays. AOR activity was improved to 20 U mg^{-1} by increasing the tungsten concentration 1000-fold (from 12 nM to 12 µM).

We are currently studying the biochemical properties of this biotechnogically relevant tungstoenzyme. Perspectively, the use of a protein production system in a related anaerobe will allow for the production of AOR enzyme variants towards deeper understanding of the enzyme and for improvement of its catalytic properties.

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Reversible RNA phosphorylation stabilizes tRNA for cellular thermotolerance

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Post-transcriptional modifications play critical roles in tRNA stability and function[1-4]. In thermophiles, tRNAs are heavily modified to maintain their thermal stability under the extreme growth temperature[5, 6]. In this study, we identified 2′-phosphouridine (U^p) at position 47 of tRNAs from thermophilic archaea[7]. U^p47 confers thermal stability and nuclease resistance to tRNAs. Atomic structures of archaeal native tRNA revealed a unique metastable core structure stabilized by U^p47. The 2′-phosphate of U^p47 protrudes from the tRNA core and prevents its backbone rotation during thermal denaturation. In addition, we identified the *arkI* gene, which encodes an archaeal RNA kinase responsible for U^p47 formation. Structural studies revealed that ArkI has a non-canonical kinase motif surrounded by a positively-charged patch for tRNA binding. A knockout strain of *arkI* grew slowly at high temperature and exhibited a synthetic growth defect with depletion of the other tRNA-modifying enzyme. We also identified an archaeal homolog of KptA as an eraser which efficiently dephosphorylates U^p47 *in vitro* and *in vivo*. Taken together, our findings show that U^p47 is a reversible RNA modification mediated by ArkI and KptA that fine-tunes the structural rigidity of tRNAs under extreme environmental conditions.

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Anti-biofilm and anti-adhesive molecules from Antarctic marine bacteria

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The increasing impact of bacterial biofilms on human health[1] increases the interest in the development of new approaches to prevent surface adhesion and biofilm formation[2]. A viable approach should target the adhesive properties without affecting bacterial vitality to avoid the appearance of escape mutants. In the research of new anti-biofilm agents, the exploitation of biodiversity is the main road, and microorganisms able to thrive in harsh conditions, like in Antarctica, represent an untapped reservoir of biodiversity[3]. Antarctic bacteria living in adverse environmental conditions developed unusual survival strategies, such as an antagonistic activity that reduces the presence of competitive microorganisms. Such behaviour is necessary when nutrients are limited or difficult to uptake, indeed, a preliminary characterization of several molecules isolated from Antarctic bacteria revealed that these compounds display anti-biofilm activity[4,5,6,7].

We report the identification and purification of new anti-biofilm and anti-adhesive molecules produced by different Antarctic marine bacteria active against *Staphylococcus epidermidis*, an important opportunistic pathogen in infections associated with medical devices[8].

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A system-level perspective on heat shock response in *sulfolobus acidocaldarius*

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Temperature stress is a crucial environmental parameter for all living organisms, but is poorly studied in Archaea. For *Sulfolobus acidocaldarius*, growing optimally at 75°C, heat shock response is characterized by an upregulation of heat shock proteins (HSPs), mainly chaperones. However, it is unknown how this temperature-sensing and molecular regulation of HSPs is established. This work aims to address these questions by unravelling heat shock response using a system-level perspective.

Pulse-labeling experiments indicated that, upon heat shock, *de novo* RNA and protein synthesis is decreased in *S. acidocaldarius*, but not completely shut down. Further, transcriptomic (RNA-sequencing) and proteomic (mass spectrometry) analyses demonstrated a global, fast transcriptional response and slower translational reprogramming. Differential expression analysis confirmed significant effects for many transcripts and proteins associated with chromosome organization, DNA topology and DNA import/repair and a downregulation of genes encoding the DNA replication and cell division apparatus.

A direct correlation between transcriptional expression and translational production was not evident for all genes, suggesting the existence of post-transcriptional regulatory processes. This hypothesis was further investigated for the gene encoding a thermosome subunit, one of the most important HSPs, which harbors a 5'-untranslated region (5'-UTR). A 5'-UTR deletion strain was constructed; qRT-PCR and Western blotting confirmed the importance of this 5'UTR-region as a determinant for correct HSP protein levels at the optimal growth temperature its heat-shock responsive upregulation. To our knowledge, this is the first demonstration of a leader-associated, temperature-responsive post-transcriptional regulation in an archaeal host.

Pushing *protein-tags* on the boundaries: new thermostable tools for expanding biotechnological applications

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The specific labelling of proteins in recent years has made use of self-labelling proteins, such as the SNAP-*tag*® and the HaloTag®[1,2]. These enzymes, by their nature or suitably engineered, have the ability to specifically react with their respective substrates, but covalently retaining a part of them in the catalytic site after the reaction[1,2]. However, due by their mesophilic origin, these *protein-tags* have the disadvantage to be employed only in mild reaction conditions and in mesophilic model organisms[3].

The growing demand to use recent technologies at very extreme conditions (in thermophilic bacteria and archaea), such as the *in vivo* CRISPR-Cas immune systems, leads us to search for new *protein-tags* with marked thermostability and activity at high temperatures. In this regard, in recent years our studies focused on the identification, characterization and engineering of AGTs from (hyper)thermophilic microorganisms[4], in order to develop new thermostable SNAP-*tags* to be applied as biotechnological tools in *in vitro* harsh reaction conditions[3,4], as well as in *in vivo* heterologous expressions in thermophilic model organisms (*Thermus thermophilus*[3] and *Sulfolobus islandicus*[5]). Recently, starting from the engineered variant of the *Sulfolobus solfataricus* OGT used as thermostable SNAP-*tag* (the H^5 mutant[3]), we furtherly engineered this enzyme achieving, for the first time, a thermostable version of the CLIP-*tag*®[6], which shows a modified substrate specificity respect to the SNAP-*tag*®, thus allowing a simultaneous multi-protein labelling in extreme reaction conditions.

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Hydrogenotrophic microbial enrichments from an alkaline hydrothermal spring driven by serpentinization, prony bay, new caledonia

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The shallow Prony Bay Hydrothermal Field (PBHF) combines characteristics of both terrestrial and marine ultramafic systems. Therefore, it presents a unique opportunity to study life in a serpentinisation context. The intertidal carbonate chimneys emit alkaline, hot, and anoxic fluids enriched in H2, CH4, and abiotically produced organic compounds [1]. These reduced compounds constitute a potential energy source for the microbial communities colonizing the chimney [2], while the ambient seawater provides oxidants for microbial respiration. Metagenomics data suggest that primary production is based on hydrogenotrophy [3], but experimental evidence on the metabolic functioning of associated communities is lacking so far. Here, we produce a first series of enrichment cultures from PBHF and test the influence of the terminal electron acceptor (TEA) on community structure. Stirred, H2-sparged bioreactors with synthetic hydrothermal fluid at 35°C/pH 10 were used to test four different TEA growth conditions (O2, NO3-, SO42-, soluble DIC or DOC) with 5mM formate and 1mM acetate as the sole carbon source. Growth was monitored over a period of 3 weeks by cell-counting and measuring metabolic activity (HPLC, CPG). Microbial diversity was initially assessed by 16S rRNA analysis using both an amplicon-based and gene capture approach. Subsequently, community structure and function was investigated in depth via metagenomics and metatranscriptomics. Consistent growth (up to 108 cells/mL) was obtained in each condition with significant enrichment of the targeted metabolic groups. Highly abundant ASVs of the genus Serpentinimonas were found in cultures with O2 and NO3-. The genus Anaerobacillus was very present in cultures with NO3- and SO42-. We observed community structuring according to the TEA and enrichment of microorganisms previously never cultivated, such as Actinobacteria (candidate class OPB41) and the two archaeal phylotypes TCMS and LCMS, specific to serpentinized media. Metagenome Assembled Genomes (MAGs) of most of the dominant members of the consortia could be reconstructed. We will discuss implications for the functioning of PBHF.

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Application of metagenomics on MICROEukaryotes living in melting alpine and polar snow

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The Cryosphere covers up to ~43% of Earth´s surface[1]. Few eukaryotic microbes thrive in such extreme environments like melting mountainous or polar snowpacks. Snow algae serve as the main photoautotrophic producers of organic matter, while fungi and bacteria fulfil an indispensable role as decomposers. In this contribution, we will present results from two recent metagenomic projects, where Illumina MiSeq 2x300 bp sequencing was applied on macroscopically visible blooms (red, green, orange, pinkish, golden-brown snow) in mid-latitude (Austria, Czech Republic, Slovenia, Sweden, Switzerland)[2,3] and polar regions (Greenland Ice Sheet, Svalbard)[3]. The aim was to get insights into cryoflora bloom community structures, infer geographic (cosmopolite *vs.* local), and altitudinal distribution (above *vs.* below timberline) of main algal haplotypes. Furthermore, possible algal co-occurrences with fungi, and altitudinal correlation of OTUs richness was tested. For microalgae, the hypervariable ITS2 rDNA marker provided sufficient resolution for OTUs/ASVs assignment on species level. This was complemented by conservative 18S rDNA metabarcoding for giving an overview on genus or higher rank levels. Critical steps when designing eukaryotic community studies[4,5] are addressed, including „universal“ primer selection, integration of unique indices (to recognize index-switching artefacts) or reference sequences generation by Sanger. This helped to discover undescribed but not endemic species and facilitated morphologic characterisation. This case study demonstrates how to optimize the description of eukaryotic extremophilic communities.

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Novel proteins involved in the uv-stress response of *sulfolobus acidocaldarius*

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Upon UV-light exposure, hyperthermophillic model organism *Sulfolobus acidocaldarius* forms species-specific cellular aggregates depending on the Ups-pili[1]. Within these aggregates, the cells can exchange DNA using the Ced DNA importer[2], which subsequently allows DNA repair via homologous recombination.

A recent study using machine learning to analyze diverse sets of RNA-seq data, revealed several so-called iModulons[3]; groups of similarly regulated genes. One of these iModulons is related to the UV-stress response and contains five so far uncharacterized genes. We hypothesized some of these genes to be part of a not yet identified DNA exporter. In this study, we created deletion mutants of the 5 genes of interest and performed cell aggregation experiments, DNA exchange assays, and survival rate assays after UV-light exposure. Of the 5 genes of interest, one encodes a type IV pilin subunit and was found to be essential for cellular aggregation, we therefore hypothesize it to be part of the Ups machinery. Another of the genes is predicted to be a transcriptional regulator. The rest of the genes do so far not seem to be involved in cellular aggregation or DNA transport. The hypothesized DNA export system therefore remains elusive.

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Novel Chaperonin from the thermophilic Thaumarchaeote *Nitrosocaldus cavascurensis*: Recombinant expression and functional characterization

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Currently, our group is using synthetic biology to clone and express chaperonins (CPNs) in very deeply branching archaeal taxa. We describe the expression and characterization of a CPN complex from *Nitrosocaldus cavascurensis* (NCAV), a member of the Thaumarchaeota which forms one of the major clades of the TACK superphylum. Genome analysis of this extremely thermophilic ammonia oxidizing NCAV showed a CPN encoded from a conserved position within the urease operon of these strains. The CPN gene from NCAV was found to be a member of a third class of CPNs described by us as having a novel lid closure mechanism and representing a potential branch point of the two widely recognized groups of CPNs[1]. Here we test an hypothesis that the CPN60 is a specialized chaperone involved in protection or de novo folding of the urease enzyme. The clone encoding the NCAV CPN60 gene was expressed in *E. coli* purified using ion exchange chromatography and sucrose gradient zone gradient ultracentrifugation. We carried out refolding and assembly of Jack Bean Urease, which is closely related to the archaeal ureases. The function of the urease in ammonia oxidizing Archaea is to exploit urea in ammonia depleted settings as a fallback nutritional strategy to supply ammonia via hydrolysis of urea to CO_2 and ammonia. The prototype structure of this group of CPNs has biochemical deficits compared to group II CPNs found in both Archaea and Eukaryotes, namely lack of the nucleotide sensing loop and no allosteric negative cooperativity between the rings allowing uncontrolled ATP hydrolysis. We are exploring the structure of the NCAV CPN and preliminary results with CryoEM show that the complexes formed include 14-mers as well as 16-mers, a result that has not been reported previously.

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Extremozymes for xylan-derived products production

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Agro-industrial lignocellulosic residues have received increasing interest as a renewable source of functional ingredients [1]. For lignocellulosic material fractionation, non-eco-friendly processes are usually employed which allow reaching good yields in terms of hemicellulose extraction. However, those methodologies also produce undesired by-products, which should be removed prior XOS final application.

In this regard, we developed a mild alkali treatment followed by enzymatic hydrolysis at 40ºC which reached hemicellulose extraction yields equivalent to harsh treatments [2]. Nevertheless, our recent research has highlighted the importance of thermophilic enzymes in this process. Hemicellulose extraction yields increased to almost 100% when the process was performed at 65ºC with no degradation products.

Therefore, finding new thermophilic endo-xylanases, which also show halophilic and/or alkalophilic properties (for avoiding neutralization and washing steps after alkali treatment) would entail a considerable advantage for the overall optimization of XOS production. With this aim, we carried out an enriched culture using wheat straw as inoculum and carbon source. Sterile Tinteiro thermal-alkaline spring water (Ourense) was used as solvent and source of mineral micronutrients. Positive strains were selected by xylan-agar plate and Congo-red staining and identified by 16S gene sequencing.

A microorganism characterized as *Neobacillus* sp. was isolated and identified as endo-xylanase and beta-xylosidase producer, whose properties are highly favourable. Enzymes are able to work at pH 7-10, with maximal activity at 10 and 50 ºC. However, in depth enzyme characterization should be performed before industrial XOS production.

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Bacterial nano-compartments from the radation resistant bacterium, *Deinococcus radiodurans*, their function in the cellular response against stress

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Bacterial nano-compartments are known to exist in bacteria. We have studied the electron dense granules (EDG), from the radiation resistant bacteria *Deinococcus radiodurans* and the involvement of both DNA-binding proteins Dps1 (*dr2263*) and Dps2 (*drb0092*) [1-3] on these cellular substructures. *Dr*Dps are ferritin-like proteins, with the ability to store Mn and Fe as well as to bind/protect DNA under *in vitro* conditions [3]. Formation of EDG in *Dr*Dps knockout mutants is abolished, suggesting that these proteins play an important role on the formation and regulation of these bacterial nano-compartments. Using X-ray fluorescence nano-imaging data (ID16A-NI, ESRF), we have investigated the metal content in these nano-compartments, and our results showed that these are element-rich regions, namely with phosphorous, calcium and manganese [4]. Therefore, these nano-compartments act as element-rich regions under control conditions, which are triggered to release the different elements when cells are subject to stress [4]. In order to increase our molecular and structural insights on these regions, we applied for an ACCESS Instruct-Eric proposal to perform STEM analysis coupled with Electron Dispersive X-ray Spectroscopy (EDS) on the *Dr*Cells. We will present our recent results, where we will show the details of these nano-compartments regarding its heterogeneous metal composition across different cells.

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***Funding acknowledgements*:**
INSTRUCT-ERIC-Access Proposal, PID: 19879- „Unveiling the nature of cellular nano-compartments – electron dense granules by cryo-STEM“, Weizmann Institute Electron Microscopy Unit
Fundação para a Ciência e Tecnologia, Project- PTDC/BIA-BQM/31317/2017, Metals in the radiation resistance bacterium *Deinococcus radiodurans*, its role in protection up to future applications.
European Union's Horizon 2020 research and innovation programme under grant agreement No 857203**-** IMpaCT - Imaging life from Molecules to cells - building knowledge on Cryo-electron microscopy methodologies at ITQB NOVA – www.itqb.unl.pt/impact.
The X-ray fluorescence imaging experiments (ESRF proposal LS3037) were performed on beamline ID16B at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. We are grateful to Local Contact, Remi Tucoulou at the ESRF for providing assistance in using beamline ID16B.

The structure and activities of the archaeal transcription termination factor Eta detail vulnerabilities of the transcription elongation complex

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Transcription must be properly regulated to ensure dynamic gene expression underlying growth, development, and response to environmental cues. Regulation is imposed throughout the transcription cycle, and while many efforts have detailed the regulation of transcription initiation and early elongation, the termination phase of transcription also plays critical roles in regulating gene expression. Transcription termination can be driven by only a few proteins in each Domain of Life. Detailing the mechanism(s) employed provides insight into the vulnerabilities of transcription elongation complexes (TECs) that permit regulated termination to control expression of many genes and operons. Here, we describe the biochemical activities and crystal structure of the superfamily 2 helicase Eta, one of two known factors capable of disrupting archaeal transcription elongation complexes. Eta retains a twin-translocase core domain common to all SF2 helicases and a well-conserved C-terminus wherein individual amino acid substitutions can critically abrogate termination activities. Eta-variants that perturb ATPase, helicase, translocase, and termination activities identify key regions of the C-terminus of Eta that, when combined with modeling Eta-TEC interactions, provide a structural model of Eta-mediated termination guided in part by structures of Mfd and the bacterial TEC. The susceptibility of TECs to disruption by termination factors that target the upstream surface of RNA polymerase and potentially drive termination through forward translocation and allosteric mechanisms that favor opening of the clamp to release the encapsulated nucleic acids emerges as a common feature of transcription termination mechanisms.

Cazyme discovery from geothermal environments for biotechnological applications

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Novel thermophilic glycosidases, showing uncommon intrinsic stability to pH extremes and temperatures >80°C[1] are very promising candidates for the biotransformations and biotechnological application requiring extreme reaction condition as for lignocellulosic materials in second-generation biorefineries. We report here a metagenomic approach aimed to search novel Carbohydrate active enzymes (CAZymes)[2] within the hyperthermophilic microbial communities populating geothermal sites. The metagenomic analysis of the microbial consortia in two neighboring mud/water pools in the solfataric field of Pisciarelli (Naples, Italy) that differ in temperature and pH (Pool1 T=85°C and pH 5.5; Pool2 T=94°C and pH 1.5) was performed. Moreover, to identify enzymes to be exploited in the conversion of lignocellulosic biomasses for second-generation biofuels, we enriched in-lab Pool1 community to select microorganisms able to grow on different plant biomasses.

The analysis of metagenomic data revealed a high abundance of CAZymes in the solfataric samples. In particular, within the CAZymes present in Pool2, we identified and characterized a novel hyperthermostable GH5 α-mannosidase and a novel archaeal glucosidase/*N*-acetyl-glucosidase belonging to a new family of glycosidases to date not yet classified in CAZY database (www.cazy.org).

These results showed that a combined approach of metagenomic of extreme environments, in-lab enrichments, and detailed enzymatic characterization is a powerful tool to exploit natural biodiversity and obtain novel biocatalysts for industrial applications.

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Going hardkor: elucidating the genome evolution of korarchaeota

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Korarchaeota – "the early diverging archaea" – was the third archaeal phylum to be discovered and the first to be proposed without a cultured representative[1]. The lineage was originally detected in a hot spring in Yellowstone National Park, but later found to be present in many more extreme yet thermal environments on Earth[2-4].

Our knowledge of archaeal diversity, function and evolution has expanded rapidly the past decade, mainly as a consequence of large-scale utilization of genomic tools[5]. However, to date, only ~30 draft Korarchaeota genomes exist because of the inaccessibility of their natural habitats for sampling and potentially their limited diversity and abundance. As a result, many aspects about Korarchaeota biology and physiology remain enigmatic.

In the current project, we expand the Korarchaeota with five new partial genomes obtained from sediment collected from the terrestrial hot spring Little Hot Creek (California, USA) and the Taketomi Island shallow submarine hydrothermal field of the Southern Ryukyu Archipelago (Japan). Using phylogenomic analyses we not only accurately determine the placement of Korarchaeota in the archaeal branch of the Tree of Life, but also the internal topology of the phylum. Furthermore, using the information enclosed in the genomes we describe how living in different marine and terrestrial thermal habitats shaped the genomic landscape of the Korarchaeota and how patterns of gene loss and gain shaped the transition between these habitats.

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Archaeal histones specify the site of foreign DNA integration at CRISPR loci

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CRISPR systems confer adaptive immunity against viruses and other mobile genetic elements to a broad range of archaeal and bacterial species. CRISPR systems adapt to invasive mobile genetic elements by incorporating short fragments of foreign DNA (referred to as spacers) into the CRISPR array of the host. Remarkably, new spacers are incorporated into CRISPR loci in a directional manner with integration almost invariably taking place at the repeat sequence located immediately downstream of the leader DNA rather than at identical repeats found throughout the CRISPR array. *In vitro*, we and others have shown that Cas1-Cas2 integrase complexes often integrate spacers indiscriminately at each repeat of the CRISPR array, suggesting that host factors normally ensure that new spacers are added selectively at the leader proximal repeat *in vivo*. I will present exciting findings supporting a key role for histones in directing spacer integration in archaea. We observe histones binding to leader DNA of *Pyrococcus furiosus* CRISPR arrays *in vivo* and show that new spacer integration is inhibited in strains lacking one of the two histone proteins. Moreover, we demonstrate that purified histones are sufficient to direct spacer integration to the leader-proximal repeat *in vitro*. Our results indicate that histones coordinate the formation of temporally-ordered and heritable immunological memory used to prevent recurrent infections by archaeal viruses and plasmids.

A platform for identifying functions associated with Unannotated proteins in s. Acidocaldarius

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Archaea, the third branch of the phylogenetic tree, comprises unicellular organisms that are often localized to niches inhabitable to many organisms and are therefore referred as extremophiles. They accomplish this through exploiting these extreme environments using unique biochemistries, which are often relevant to biotechnology. Despite their biochemistry being poorly understood, their enzymes (extremozymes) and certain metabolites find application in industries for food, feed and textile processing as well as in scientific research.[1] As many manufacturing processes are currently being redesigned with environmental sustainability in mind, novel enzymes could replace traditional chemical processes as we transition towards greener manufacturing processes.[2]

Traditional biochemical approaches to elucidate enzymes involved in metabolic pathways have provided us with vast amounts of data but often require laborious time-consuming methods and are suitable only to a specific organism. With the horizons for the discovery of new microbes having been broadened by the advent of metagenomics, we find that there is a lack of efficient strategy for elucidating biosynthetic pathways in the newly identified organisms. With the help of our in-house multi-omics workflow, we demonstrate that high-throughput growth media screens when combined with metabolomics and proteomics could reveal patterns in enzyme abundances revealing the chemistries that are associated with them.

We perform a screen of about 180 different growth media conditions for *S. acidocaldarius* and identify patterns in growth for groups of compounds like acids, alcohols, amines etc. A closer examination of the enzymes enriched when grown on specific compound groups reveal enzymes that could have roles in the metabolism of compound groups. Combining with the metabolomics data, we are able to predict the functions to some of the enzymes whose functions are yet to be uncovered.

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Metagenomics-based culturomics of hypersaline habitats

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Hypersaline environments are represented by aquatic habitats (saline lakes, salterns) and saline soils, as well as other systems such as salt mines, salted food, etc…Early metagenomic studies on Spanish salterns permitted us to determine the microbial diversity of ponds of these extreme habitats. The prokaryotic diversity of the crystallizers (ponds with saturated salts) is represented by haloarchaea (mainly *Haloquadratum* and *Halorubrum*), nanohaloarchaea and halophilic bacteria (*Salinibacter*). However, a greater prokaryotic diversity is present in intermediate salinity ponds, in which a large percentage of the microbiota had not been cultured under laboratory conditions. The success on the isolation of the new moderately halophilic bacterium *Spiribacter salinus*[1], based on previous metagenomic studies on a Spanish saltern, representing up to 12-15 % of the total prokaryotic population of the intermediate-salinity ponds, aimed us to carry out further culturomic studies, taking into consideration the previous metagenomic data. The use of a wide variety of media with different compositions and salinities as well as distinct culture conditions, have permitted us to isolate and characterize several new groups of archaea that have been shown to be relatively abundant on the intermediate salinity ponds. We will show data concerning some of these representative new taxa, such as two new species of the haloarchaeal genus *Halonotius*[2], that present the complete cobalamin biosynthesis gene cluster, suggesting that species of this genus could play a relevant ecological role, two new species of the new genus *Halosegnis*[3], that have a worldwide geographical distribution and relatively abundance (up to 8 % in habitats with intermediate salinities), as well as other haloarchaeal taxa recently isolated.

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Composition and function of metabolically active chemosynthetic microbial biofilms at deep-sea hydrothermal vents

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Deep-sea hydrothermal vents occur where heated fluids, generated by the reaction of seawater with hot magma and enriched in reduced chemical species, are released at the bottom of the ocean. This process creates physiochemical gradients which biology can exploit. 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 active microbial biofilm communities collected from distinct biological regimes characteristic of the East Pacific Rise (9°50 N, 104°17 W) vent system. Biofilms from native substrates (*e.g.*, animal surfaces, basalts, sulfides) and associated with tubeworm (*Riftia* and *Alvinella*) and mussel (*Bathymodiolus*) colonies were compared to newly-formed biofilms gathered via the deployment of microbial colonization devices. 16S rRNA-based analyses revealed that *Sulfurimonas*, *Sulfurovum* and *Arcobacter* genera dominated newly-formed biofilms across all biological regimes (Epsilonproteobacteria constituted 70-99% of sequences, $\bar{x}$ = 91%), while established biofilms associated with invertebrates were more diverse. Statistical analyses using fluid chemistry data from each sampling site suggest that community composition is significantly impacted by biofilm age, temperature, sulfide concentration and pH, and to a lesser extent, locality. Metatranscriptomic and metaproteogenomic analyses were used to establish the biofilm functional diversity. The discussion will focus on the type and abundance of transcripts and proteins related to central metabolism, energy yielding processes and DNA exchange and recombination.

Microbial life in subsurface planetary analogues in Iceland

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With their harsh conditions, Icelandic lava tubes present an excellent analogue for Martian subsurface environments, giving insight into bioprocesses required for life in caves during Mars' potentially habitable distant past, and thus giving clues as to biosignatures that may be conserved on geological timescales. Coupled with in situ X-ray fluorescence measurements of the substratum, speleothems and their associated microbial mats in several Icelandic lava tubes were sampled for eDNA analysis by 16 rRNA gene-based community analysis, and from one cave, samples were taken for cultivation and biochemical and genomic characterisation of bacterial isolates from rock wall and ice. The microbial community composition was found to be majority *Proteobacteria* with *Ralstonia*, *Caulobacter*, *Cupriavidus*, and *Corynebacterium* accounting for the majority of the genera detected in the eDNA analysis. The cultivated bacteria comprised a diverse collection, including genera such as *Massilia, Sphingomonas, Polaromonas, Arthrobacter, Brevundimonas, Flavobacterium* and *Pseudomonas*. The ability to grow with various substrates and utilise a range of pathways was assessed, with a particular focus on the solubilisation of inorganic phosphate, a process essential for the supply of bioavailable phosphorous to other organisms. Multiple species were found to be phosphate solubilising on solid and liquid. *Pseudomonas gessardii* strains exhibited the fastest solubilisation rate, whilst various *Arthrobacter* species also showed the ability to rapidly solubilise phosphate alongside growth. In contrast to a visible ability to solubilise phosphate on solid media, *Sphingomonas faeni* isolates showed little evidence of solubilisation in liquid media. A subset of these isolates were selected for whole genome nanopore sequencing, so as to give an overview of the genetic makeup of these extremophilic microbes and give insights as to some of the biogeochemical pathways that are required for life in these harsh environments.

Evolutionary pathway of *acidihalobacter* from a halophile to an acidihalophile

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Acidihalobacter is a unique genus of iron- and sulfur- oxidising acidophilic known for its ability to oxidize pyrite minerals in the presence of elevated chloride ions[1,2]. Currently, four species of the *Acidihalobacter* genus have been identified: *A. prosperus*, *A. yilgarnensis*, *A. aeolianus* and *A. ferrooxydans*. Previous research has focused on the genetic arsenal that allows *Acidihalobacter* to cope with chloride, metal and oxidative stress[3,4]. In this study we investigated the genetic repertoire that has enabled the *Acidihalobacter* genus to cope with acidic stress. Phylogenetic analysis shows that the *Acidihalobacter* genus roots to the Chromatiales class consisting of mostly halophilic microorganisms. We propose gene gain events that enable the *Acidihalobacter* genus to cope with acid stress. Potential acid tolerance mechanisms include multiple potassium transporters, chloride/proton antiporters, glutamate decarboxylase system, arginine decarboxylase system, urease system, *slp* genes, squalene synthesis, and hopanoid synthesis. Some of these genes are hypothesized to have entered the *Acidihalobacter* via vertical decent from an inferred non-acidophilic ancestor, however horizontal gene transfer from other acidophilic lineages is probably responsible for the introduction of many acid resistance genes.

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Polyphasic characterization of two newly isolated thermal cyanobacteria of the *leptolyngbya* genus

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The biodiversity of the Euganean Thermal District (Padova, Italy) has been recently investigated considering both the microbiota that colonizes the therapeutic muds during the process for its production in several Spas[1] and the one found in natural hot springs in the surrounding area of the Regional Park. Cyanobacteria represent one of the main phyla found in either artificial or natural conditions. Exopolysaccharides (EPS) and glycolipids extracted from cyanobacteria isolated from this environment have already been tested for their anti-inflammatory activity *in vitro* and *in vivo*, highlighting the importance of studying the biodiversity that enriches these environments. Two filamentous cyanobacteria have been isolated from samples collected from hot springs, called respectively Euganean Thermal Springs (ETS) strains ETS13 and ETS31. They have been identified as new stains belonging to the *Leptolyngbya* genus through optical and electron microscopy analysis, evaluation of the pigment composition, 16s rRNA sequence, and NGS analysis of the whole genome. Interestingly, ETS31 was discovered to perform type 3 chromatic acclimation, a photoreversible process that allows to maximally absorb the light spectrum available through the modification of phycoerythrin and phycocyanin levels[2]. As for ETS13, far-red light photoacclimation is achieved when grown using far-red light, determined by the rearrangement of the photosynthetic apparatus including a shift from chlorophyll *a* to chlorophylls *d* and *f*[2]. This acclimation could be useful in far-red enriched environments such as microbial mats. Working in laboratory with *Leptolyngbya* strains is challenging due to its filamentous and slow-growing forms[3], however their study is fundamental for the discovery of new secondary metabolites that might possess therapeutic properties and for a deeper knowledge of different acclimation mechanisms.

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Argonaute tinder: Searching for protein partners of Argonautes from hyperthermophilic Archaea for functional and applied studies

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Argonaute are key enzymes of the RNAi (RNA interference) pathway in eukaryotes performing RNA-guided RNA targeting. Small RNA guides are processed from exogenous (e.g. virus) or endogenous (e.g. non-coding RNA) RNA sources and are loaded onto eAgos (eukaryotic Argonautes) with the help of other RNAi-specific proteins. Once loaded with a guide, catalytically active eAgos slice complementary RNA targets themselves, while catalytically inactive eAgos only bind the target molecule, resulting in recruitment of other cellular proteins which induce post-transcriptional or transcriptional silencing events. The cooperation between eAgos and their partner proteins leads to the regulation of a plethora of different cellular functions, ranging from virus defense over regulation of specific genes up to chromosome segregation.

Homologs of both active and inactive Argonautes are readily detected in prokaryotes, with the closest relatives to eAgos being found within within the archaeal domain (here referred to as **arc**Agos), while other RNAi-like proteins have not been identified in prokaryotes. The few studied arcAgos were shown to often use DNA or RNA guides for DNA targeting which, in some cases, led to host defense. However, the *in vivo* function in an uninfected cell, functional analogies with eAgos, as well as the identity of native guides and targets remain largely unexplored. Pressing questions of how arcAgos, especially the inactive ones, acquire guides and if arcAgos crosstalk with other cellular enzymes to elicit their biological function, still remain. Here, we characterize the cellular interaction network of selected active and inactive arcAgos in their native hosts. arcAgo hosts were specifically chosen to be genetically tractable, allowing for purification of the endogenous arcAgo. Protein partners of pulled-down arcAgo proteins are characterized and their interaction is biochemically investigated. Lastly, the possibility for establishing thermostable arcAgos (and their protein partners) as genome editing tools for other (hyperthermophilic) prokaryotes was evaluated.

Studying interaction partners of arcAgos in their native hosts will help to draw a comprehensive picture of their biological functions and deliver important insights into the evolutionary understanding of how deeply rooted the well-characterized roles of their eukaryotic counterparts are.

POSTER ABSTRACTS

Cross-bioaugmentation among two kuwaiti hypersaline soils

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Kuwait belongs geographically to a semiarid region and is characterized by harsh climate. High temperatures with trapped seawaters during tidal movement form hypersaline areas with NaCl concentrations reaching 4 M and higher, called the supertidal “sabkhas”. Like elsewhere, these areas in Kuwait are subjected to pollution with waste hydrocarbons. Interesting studies have been published on halophilic hydrocarbonoclastic microorganisms from hypersaline environments and our research group in Kuwait contributed to such studies[1, 2]. In this study, cross-bioaugmentation among two contaminated hypersaline areas were done to remove crude oil. Hypersaline soil samples from two “sabkhas” at northern and southern of the Kuwaiti shore of the Arabian Gulf were collected, contaminated with 5% crude oil and kept for 6 months to prepare inocla enriched with hydrocarbonoclastic microorganisms. Each inoclum was used to inoculate a freshly collected and oil-contaminated soil core of the opposite sabkha. Oil-consumption and bacterial communities were monitored periodically through 6 months. Although the inocla had the same bacterial community structure and consist mainly of *Bacillus oceanisediminis*, the results showed that cross-bioaugmentation enhanced oil-degradation in one sample (Southern) and inhibited it in the other (Northern). The numbers of hydrocarbonoclastic bacteria showed significant increases in both unbioaugmented and bioaugmented in both sabkhas soils during the course of bioremediation. The inoculated bacterial taxa failed to colonize the soils in both samples, while other Bacillus spp. were predominants. The two sabkha soil samples showed a significant self-cleaning potential in heavily oil-contamination reached 20%. It was concluded that the oil-degradation potential of the indigenous halotolerant/halophilic hydrocarbonoclastic bacteria had better effects on oil-bioremediation than cross-bioaugmentation.

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Deciphering the insights of an acrylamide degrading *Burkholderia* sp. amidases for versatile biodegradation applications

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The present work highlights the isolation and characterization of the moderately thermophilic acrylamide degrading bacteria, *Burkholderia* sp. EMB 26 via soil enrichment technique. The strain exhibited a good production of amidases, leading to effective acrylamide degradation as revealed by its genomic inference using whole genome sequencing, deciphering the pathway utilized by the bacteria for biodegradation. Further, the phylogenetic and metabolic pathways assessments were also investigated to deduce the degradation potential, virulence factors and pathogenicity. The scanning electrochemical microscope studies demonstrated that EMB 26 decomposed acrylic polymer, which was subsequently validated utilizing whole-cell biodegradation to eliminate 14 mM (1000 ppm) of acrylamide completely within four days. Further, to elucidate the potential role of amidases in acrylamide degradation, purification and recombinant studies were carried out. Amidases play a crucial role in degrading acrylamide into the corresponding acid. The work establishes that our strain was able to utilize the degraded metabolite, i.e., acrylamide, amide substrate and liberated ammonia as carbon and nitrogen sources, respectively, along with a key role in styrene polymer degradation. The work also deals with aliphatic amidase purification by anion exchange chromatography yielding upto 19-fold with 38 kDa molecular weight. The biochemical characterization and kinetic parameters showed the highest affinity towards acetamide. The structural-functional analyses by fluorescence and far UV-CD spectroscopy elucidated the structural insights of the enzyme. The study further delved into a heterologous expression of unique nicotinamidase. The enzymatic characteristics of the recombinant amidases have also been investigated, which marked its broad substrate specificity. Thus, the degradation potential of *Burkholderia* sp. EMB 26 holds a promising future for the degradation of various acrylic-based polymers and hydrocarbons.

Biochemical insights into the radiation resistance of the extremotolerant bdelloid rotifer *Adineta vaga*

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All aerobic life forms experience oxidative stress which gets magnified upon exposure to obnoxious conditions like desiccation, ionizing radiation or freezing. Among eukaryotes, rotifers of the class Bdelloidea are one of the few metazoan clades possessing an exceptional resistance to those different stresses. Here we present the results of our study investigating the molecular basis of radiation tolerance of our model rotifer system *Adineta vaga*. First, we observed a strong correlation between radiation survival and a high intracellular ratio of manganese to iron, as reported for the radiation-tolerant bacteria *Deinococcus radiodurans*. However, in other radiation-tolerant eukaryotes, including tardigrades and amoeba, we did not detect such high manganese to iron ratios. We further evaluated the impact of manganese and iron on radiation resistances through manganese-depletion and iron-toxicity assays, disclosing a stronger radiation sensitization in *A. vaga* upon manganese starvation and on increasing iron levels. Upon characterizing protein oxidation levels following radiation exposure, we investigated the role of manganese as an antioxidant and radio-protectant in the bdelloid rotifer *A. vaga*. We also compared manganese coordination (understanding the nuclear centers interacting with manganese centers) in *A. vaga* to radiation-tolerant and radiation-sensitive prokaryotes and eukaryotes through EPR spectroscopy, showing distinct manganese coordination in *A. vaga* as compared to *D. radiodurans.* Briefly, *D. radiodurans* as published before highlights two distinct molecular configurations of manganese where nitrogen, oxygen and phosphate centered species are observed to be the major contributors towards Mn speciation, while *Adineta vaga* on the other hand seems to contain majorly one configuration with a major interaction with oxygen and phosphate centered species. Since manganese antioxidant complexes are known to be potent superoxide radical scavengers, we performed an in-gel superoxide scavenging assay to understand superoxide scavenging across different extremotolerant and non-extremotolerant organisms. Briefly, we highlight an expansion of superoxide scavenging complexes within *A. vaga* where a subset of such complexes is observed to be manganese dependent, while *D. radiodurans* seems to be majorly dependent on manganese. Overall, this study identifies an interesting phenomenon of convergent evolution where manganese containing complexes seem to play a major role in radiation tolerance.

Cyanobacteria under simulated non terrestrial conditions: from astrobiology to biotechnological relapses

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Many terrestrial-like exoplanets were found orbiting the Habitable Zone (HZ) of M-dwarf stars, stars with little emission of light in the visible and high emission in the far-red and near-infrared. Is it possible then for oxygenic photosynthesis (which works using visible light) to function on planets orbiting these stars and to produce atmospheric and surface biosignatures, biological traces that could reveal life beyond our Solar System? The discovery of cyanobacteria able to perform the Far-Red Light Photoacclimation (FaRLiP)[1] and thus able to utilize far-red to photosynthesize and grow, could answer this question. We performed experiments on cyanobacteria able or unable to perform FaRLiP, exposing them to simulated M-dwarf , solar and far-red light spectra and analysing their growth and acclimation through innovative instrumentation[2,3]. The results show the impact that cyanobacteria could have in a terrestrial atmosphere under a simulated M-dwarf light spectrum and our possibilities to detect it from remote. Moreover, the results are potentially applicable outside the astrobiological field, to find light regimes, e.g. for industrial cultivation, which offer a compromise between growth of the cyanobacteria and energy usage due to electrical consumption.

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The energy conserving hydrogenases Ech2 in *Thermoanaerobacter kivui* is not essential for acetogenic growth on H_2 + CO_2 but essential in CO metabolism

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Thermoanaerobacter kivui is one of few promising thermophilic acetogenic bacteria for conversion syngas (H_2, CO_2 and CO) into bulk chemicals. The reduction of CO_2 and CO occurs via the Wood-Ljungdahl pathway as terminal electron accepting pathway. Two membrane-bond energy conserving hydrogenases (Ech1 and Ech2) are proposed to be part of the energy and redox metabolism of *T. kivui* [1]. Their assumed function is the reduction of protons to molecular hydrogen (H_2) with electrons derived from the oxidation of reduced ferredoxin (Fd_{red}) during growth on H_2/CO_2. Since Ech1 and Ech2 are the only putative coupling sites, the redox potential difference is utilized to build up an electrochemical membrane potential which can be converted into ATP by ATP-synthase [2]. In heterotrophic metabolism, the complexes may exploit the membrane potential to provide Fd_{red} to the cells.

Here, we addressed the question why *T. kivui* has two Ech complexes, and whether they have distinct physiological functions. Therefore, we generated a deletion mutant of the *ech2* gene cluster based on a recently developed genetic toolbox for *T. kivui* [3]. Growth experiments with *T. kivui* wild type and the Δ*ech2* mutant interestingly show a similar growth rate of both strains on glucose (0.42 h^{-1} *vs.* 0.44 h^{-1}), mannitol (0.36 h^{-1} *vs.* 0.36 h^{-1}) and H_2/CO_2 (0.53 h^{-1} *vs.* 0.50 h^{-1}). Resting cell experiments show that Δ*ech2* does not produce acetate, formate and H_2 when incubated with CO[4]. The wild type but not Δ*ech2* could be adapted to growth on CO by serial transfer. In conclusion, the Ech2 complex of *T. kivui* is essential for CO metabolism but not for growth on glucose, mannitol and H_2/CO_2. Further qPCR analyses will provide insight into regulation of H_2 metabolism in both the wild type and the mutant strain. We are currently investigating the role of Ech1 in *T. kivui*.

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Identification of *Spiribacter* species with different frequencies and functions in lakes of central Iran with salt and oil concentration gradient

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Abstract – *Spirobacteria* are known as moderate halophile in lakes associated with moderate salinity [1]. In general, carbohydrate metabolism was the same in wetlands, but energy metabolism was different in the third wetland, in which both salinity and oil pollution were obtained low and different. Due to the effect of environmental conditions on the potency of genus *Spirobacteria* [2], there is the potential to be isolated from the environment using the culture-dependent methods. This paper aims to investigate metagenomic studies on lakes in central Iran (Beheshte-Masoumeh Qom) under different salinities near the first oil field. After determining the specific geographical, the location of three point of lake were measured with salinity of 6%, 3% and 1% of the water samples. Then, the physicochemical analysis and Total Petroleum Hydrocarbon (TPH) were implemented and then the genetic content of the samples was sequenced using lumina || platform6000Novaseq. *Spiribacter* genus belongs to the family *Nitrococcaceae*, within the order *Nitrococcales*, class *Gammaproteobacteria* in the phylum. *Spiribacter_soda*, *Spiribacter_Spiribacter*, and *Spiribacter_uncultured* were observed by abundances of 0.21,0.49,1.57٪ in the first lake (w_1), by abundances of 0.18% 0.37% 2.69 % in the second lake (w_2), and by abundances of 0.14% 0.12% 1.36% in the third lake (w_3). Besides, Gtdbtk software was compared due to different oil pollution and salinity of lakes. According to the obtained results, Xenobiotics biodegradation pathway of Xylene, Fluorobenzoate, Toluene, Ethylbenzene, Atrazine and Dioxin were not observed in the third lake (w_3) including less salinity and pollution, whereas they were observed in both lake w_1 and w_2. In terms of amino acid metabolism, biosynthesis of Ectoine was observed in w_1 and w_2 while the biosynthesis of Betaine was observed only in the first wetland.

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Surfactants as a tool to fight extremophilic microalgal contamination in cyanobacterial cultures

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Contamination with unwanted fast-growing microalgae is one of the current problems in large-scale microalgae cultivation, significantly when growing slow-growing species. *Coccomyxa simplex* is an extremophilic microalgae that commonly appear as contaminant in microalgal and cyanobacterial cultures. That microalga genera is able to grow in an extensive range of physic-chemical conditions, including acidic and metal contaminated waters (1). Finding physical conditions and chemical compounds with specific effects over different microalgal species could be postulated as a good strategy to decontaminate cultures of low growth microalgal species. In this sense, surfactants have been found to display toxicity to microalgae at a different intensity depending on species (2). Therefore, the use of surfactants as a potential tool to decontaminate microalgal cultures is worth to study.

The cyanobacteria “VBP01” was isolated from arid media and co-cultivated with *C. simplex* to simulate a contaminant episode. The surfactants CTAB, TMN6, NP9, X100, and SDS were tested at different concentrations to evaluate the toxicity over *C. simplex* and the cyanobacteria “VBP01”. The use of large concentrations of TMN6 and SDS resulted in a different toxicity extent in both microorganisms, with a major impact in *C. simplex* cells.

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Acknowledgements: This study was funded by the biotech company UVera and by Grant P20_00930 from the Andalusian Plan for Research, Development and Innovation, within the frame of the operational programme “FEDER Andalucía 2014-2020”.

Molecular diversity and biocatalytic prospects of haloalkaliphilic bacteria from an indian saline desert

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Deserts in India are not extensively explored for microbial diversity as compared to other deserts worldwide [1,2]. The Little Rann of Kutch (LRK), a coastal saline desert, represents a unique combination of dryland and wetland ecosystems [3]. Some recent studies have revealed the existence of novel taxa and their biotechnological potential [3,4]. However, despite of its ecological significance, LRK is not extensively explored for microbial diversity. Here, we for the first-time report on the cultivable bacterial diversity of LRK investigated by a combination of variability of media, diluents and extended growth time. This study therefore represents the extensive isolation, spatial distribution, 16S rRNA gene-based phylogeny and identification of novel taxa. A total of 87 bacterial isolates obtained from the three different study sites in LRK were further investigated based on the full 16S rRNA gene sequences. The isolates represented by 19 different genera were grouped into 44 phylotypes of the phyla; Firmicutes, Proteobacteria, Actinobacteria, and Euryarchaeota. Among these, 15 genera reported from this habitat were never reported earlier. Majority of the isolates in this study had displayed broad salt and pH tolerance. Further, six putative novel taxa were identified. Available nitrogen, pH, Organic carbon, TDS, and EC of the habitat adjudged as the significant environmental variables affecting the microbial diversity. Analysis of the geographical distribution revealed that a majority of the phylotypes had cosmopolitan distribution, followed by the saline and marine distribution patterns. While ~13% were affiliated with only LRK. The marine distribution phylotypes decreased with increasing distance from the Gulf of Kutch, suggesting their endemism to marine environments. The bacterial Isolates were screened for the extracellular proteases, amylases and CMCase. Overall, 72% isolates produced at least one of these enzymes, while many produced multiple enzymes. The Firmicutes were dominant phyla for enzyme production. The study established the taxonomic novelty and production of unique enzymes and metabolites.

Key Words: Saline desert, Bacteria diversity, Novel Taxa, 16S rDNA, Microbial enzymes

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Alkaliphilic and haloalkaliphilic bacteria from mangroves of goa as plant growth promoters

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Alkaliphilic and halophilic bacteria from mangroves of Goa were screened for plant growth promoting traits. Gram staining of the alkaliphilic and halophilic isolates revealed predominant Gram positive bacilli. On screening of the isolates for plant growth promoting traits, they showed inorganic phosphate solubilisation , silicate solubilisation, production of phosphatase enzyme, siderophore, exopolymers and Indole Acetic Acid production at high pH and salt concentration. *Bacillus* sps when inoculated in pots as axenic culture showed two fold increase in root and shoot length of *Oryza sativa* as compared to control. This study indicates that alkaliphilic and halophilic bacteria act as efficient plant growth promoter and can be used as potential biofertilizer for alkaline and saline soil.

Keywords: Alkaliphilic bacteria; halophilic bacteria; Plant growth promoting traits; Biofertilizer.

Using the endogenous CRISPR-Cas Type I-D system for genetic engineering in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*

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CRISPR-Cas is a new genetic tool, which provides a simple and efficient way to excise genetic engineering. Most Sulfolobus species possess CRISPR type I and III systems, with type I-A, I-D, III-B and III-D being the most common subtypes. Because of the thermoacidophilic environment of Sulfolobales (65 °C – 88 °C)[1], genetic manipulation with exogenous CRISPR-Cas systems, like the most known CRISPR-Cas9, are not feasible, because of their mesophilic origin. Therefore, the aim of our study was to utilize the endogenous CRISPR-Cas Type I-D system of our crenarchaeotal model organism *Sulfolobus acidocaldarius*, to perform genetic engineering in this archaeon. CRISPR type I systems are PAM (protospacer adjacent motif) dependent, which require a three-nucleotide, organism specific motif at the 5' end of a target sequence. Three different PAMs were proposed for different Sulfolobus species: CCN, GTN, TCN.[2] As it was shown in previous studies, not all PAMs mediate the same DNA cutting efficiency.[3] Therefore wanted to test, which PAM has the highest efficiency in our system by performing knock out experiments on the well-studied archaeal gene *upsE* (*saci_1494*).

In our study we were able to efficiently generate knock-out mutants using CCA and GTA as PAMs in our mini CRISPR-Cas system alongside with establishing an optimized protocol, shortening the time necessary to obtain mutants in comparison with previously developed tools for *S. acidocaldarius*. Moreover, we were able to show, that the PAM CCA, which by Lillestøl et al. should not be viable in *Sulfolobus acidocaldarius*, was indeed usable for genetic engineering.

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Introducing high-pressure microfluidics For ultra-fast microbial phenotyping

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A majority of Earth's prokaryotes reside within the deep biosphere (i.e. subsurface environments) where little is known about how inherent elevated pressures impact the underground geochemistry and the inhabiting microbial communities. Traditional sample analysis procedure requires decompression, which induces uncontrolled biases during the investigations. Although some HP experimental means exist, microbiology under extreme conditions is still scarcely studied. Indeed, conventional cultivation and analysis techniques offer limited *in situ* characterization, thus narrowing the ability to investigate deep subsurface microbial communities.

Extreme Microfluidics which combines the advantages of microfluidics (*i.e.* size reduction, fast screening, *in situ* analyses, high reproducibility, *etc*.) with fluid systems used under high-pressure conditions (*i.e*. up to 700 bar), are modern tools particularly well adapted to investigate archaea living in extreme conditions such as deep environments. Indeed, they overcome previously cited limitations and propose fast screening approaches and *in situ* monitoring in real and extreme conditions, both in batch and continuous modes.

In this presentation, we will first detail the interest of this technology and the different strategies developed to manufacture and use high-pressure microreactors. Then, we will present the use of on-chip biocompatible high-pressure geological laboratories (BioGLoCs) for the culture and the study of methanogens living in deep geological environments. Eventually, we will present the use of microfluidics approaches for the fast phenotyping (determination of the optimal conditions) of a model deep-sea vents microorganism.

Recoding and limits of life: new insights on the recoded alpha-l-fucosidase from *saccharolobus solfataricus*

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Extremophilic Archaea represent nowadays a key area of research for the study of adaptations to harsh conditions and for exploring the limits of life. In extreme environments, these microorganisms might encounter sudden and reversible changes of the optimal growth conditions, and the flexibility of genetic code translation might be relevant to increase microbial fitness [1]. Translational recoding, which consists in a programmed deviation of the ribosomes from the standard translational rules, has been found in all three domains of life [2]. In Archaea, this phenomenon has been demonstrated for termination codon read-through events regulating the incorporation of selenocysteine and pyrrolysine, and programmed -1 frameshifting which allows the expression of a functional α-L-fucosidase in the crenarchaeon *Saccharolobus solfataricus* [3]. Although increasing evidences suggest that translational recoding could have relevant implications for life in extreme environments, little is still known about this mechanism in Archaea. Here we report that the increased level of fucA mRNA observed in certain growth conditions is related to translation efficiency [4]. In addition, the analysis of the expression of the interrupted gene encoding for α-L-fucosidase from *S. solfataricus* in the archaeon *S. acidocaldarius* revealed the synthesis of a full-length, fully active enzyme allowing the unequivocal identification of the frameshifting site *in vivo*.

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Biochemical and Regulatory Assessment of Hemicellulose Conversion in the Extreme Thermophile *Caldicellulosiruptor bescii*

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Bioprocessing of lignocellulose by current industrial hosts require pretreatment or coprocessing in order to convert fermentable sugars, hindering commercialization of renewable chemical production. *Caldicellulosiruptor bescii* is an extremely thermophilic bacterium (T_{opt} = 78 ºC) capable of growing on plant biomass without pretreatment. This combined with advances in genetic manipulation allow it to be a promising candidate for consolidated bioprocessing[1-2]. While its cellulolytic mechanisms are understood, few studies have investigated the hemicellulose conversion capabilities of *C. bescii*. Leveraging recent regulatory and metabolic reconstructions, the xylanolytic glycoside hydrolase (GH) inventory, regulator binding affinities, and xylose metabolism was investigated. Three transcriptional regulators control expression of 13 GHs for xylan degradation, including 4 secreted enzymes. Eight of these proteins were recombinantly expressed and biochemically characterized. Several proteins exhibited endo-β-xylanase, glucanase, and exo-β-xylosidase activity, suggesting a streamlined enzymatic inventory for biomass degradation. Furthermore, α-arabinosidase activity was found for an intracellular GH10 endoxylanase, which has not been characterized for enzymes from this family. Xylose metabolism was further studied through continuous cultures and transcriptomic analyses. Lower sugar uptake but comparable cell yields at fixed growth rate for xylose compared to glucose suggest an assimilatory role for xylose. Furthermore, xylose catabolism is controlled by two different regulators, suggesting tight control of pentose utilization in *C. bescii*. Overall, the assessment of xylanolytic activity and regulation facilitates engineering targets for further development of *C. bescii* into an industrial platform.

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Unravelling the molecular changes to the electrochemical potential in alkaliphiles as adaptation to increasing alkaline stresses

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The major challenge for bacteria living in an alkaline environment, is that the exterior is almost devoid of protons. Since these bacteria keep the internal pH close to neutral, the proton gradient over the membrane becomes inverted. This is a curious artefact, considering that alkaliphiles, like most bacteria, rely at least partly on a series of membrane-coupled redox reactions for generating ATP. The textbook version is an electron transport chain comprising of a series of reactions that enable proton translocation over the membrane, thereby building up a proton motive force, which is subsequently released by an ATP synthase. The proton motive force is composed of two factors: (1) the proton gradient over the membrane and (2) the electrochemical potential.

Some anaerobic alkaliphiles opt to change the coupling ion for its ATP synthase to sodium – this will not be discussed here. The other, more intriguing option, is to solve this issue with the electrochemical potential[1]. The electrochemical potential of a cell is result of the charge difference over the membrane, in other words, it is dependent on the molecular architecture of the cell. Thus, in order to draw conclusion on the subject, measurements of cell charge and membrane composition is required. In our studies, we researched this for the thermoalkaliphile *Caldalkalibacillus thermarum* TA2.A1[2, 3], using a chemostat setup at different pH levels: 7.5, 8.5, 9.5 and 10.5. We will elaborate on our findings, discussing general physiology and changes in cell charge, the (membrane) proteome and the lipidome.

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Extremophiles are set to play a valuable role in the european distributed research infrasatructure ibisba

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The circular bioeconomy is hailed by the European Commission, in the form of Europe's Green Deal, as a unique opportunity to reduce greenhouse gas emissions, increase resource efficiency and steward the transition to a more sustainable society. This involves a series of measures, including the deep transformation and innovation of the manufacturing sector, aimed at delivering a modern economy that is competitive, carbon-neutral, socially inclusive and a source of wealth and jobs. This transformative technological development is ensured and accelerated by research infrastructures and a major area of innovation lies within industrial biotechnology and the application of extremophiles in various industrial applications.

To advance the development of industrial biotechnology, the European research infrastructure IBISBA[1] (www.ibisba.eu) federates research facilities located in several regions across Europe, integrating R&D&I services, developing standards and digitalising operations to deliver fit-for-purpose bio-based solutions for a wide variety of market sectors.

The scientific and technological capabilities of IBISBA are configured in nodes, national organised ecosystems of research facilities with unique competences and expertise that, combined together, compile a comprehensive catalogue of top-quality integrative services. The Italian node IBISBA-IT (www.ibisba.it) occupies a well-defined position within four specific areas, including Synthetic Biology, Green Chemistry, Sustainable Bioenergy and Functional Food, and has the specific mission of developing new molecules and processes through enzyme/protein discovery, and new biotransformations and bioprocesses.

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Connectivity of Fennoscandian Shield Terrestrial Deep Biosphere Microbiomes with Surface Communities

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The deep biosphere is an energy constrained ecosystem yet fosters active[1, 2] diverse microbial communities with an estimated number of 2 to 6 × 10^{29} cells[3] that are key in biogeochemical cycling[4]. Whether microbial communities in deep biosphere groundwaters are shaped by infiltration of allochthonous surface microorganisms or the evolution of autochthonous species remains unresolved. In this study, we hypothesized that the fixed niches harboring a common core microbial deep biosphere biome[5] drive species sorting of surface microbes, thereby resulting in reduced diversities. 16S rRNA gene amplicon analyses showed that few groups of surface microbes infiltrated deep biosphere groundwaters at the Äspö Hard Rock Laboratory, Sweden, but that such populations constituted up to 49% of the microbial abundance. The dominant persisting phyla included Patescibacteria, Proteobacteria, and Epsilonbacteraeota. Despite the hydrological connection of the Baltic Sea with the studied groundwaters, infiltrating microbes predominantly originated from deep soil groundwater. Most deep biosphere groundwater populations lacked surface representatives, suggesting that they have evolved from ancient autochthonous populations. We propose that deep biosphere groundwater communities in the Fennoscandian Shield consist of selected infiltrated and indigenous populations adapted to the prevailing conditions.

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Integrative genomics sheds light on evolutionary forces shaping acidophilic lifestyle

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Extreme acidophiles thrive in environments rich in protons (pH values < 3) and often high levels of dissolved heavy metals. They are distributed across the three domains of the Tree of Life including members of the Proteobacteria. The Acidithiobacillia class is formed by the neutrophilic genus *Thermithiobacillus* along with the extremely acidophilic genera *Fervidacidithiobacillus*, *Igneacidithiobacillus*, *Ambacidithiobacillus*, and *Acidithiobacillus*. Phylogenomic reconstruction revealed a division in the Acidithiobacillia class correlating with the different pH optima that suggested the acidophilic genera evolved from an ancestral neutrophile within the Acidithiobacillia. Genes and mechanisms denominated as “first line of defense” were key to explaining the Acidithiobacillia acidophilic lifestyle including preventing protons influx that allows the cell to maintain a near neutral cytoplasmic pH and differs from the neutrophilic Acidithiobacillia ancestors that lacked these systems. Additional differences between the neutrophilic and acidophilic Acidithiobacillia included the higher number of genes copies in the acidophilic genera coding for “second line of defense” systems that neutralize and/or expel protons from cell. Gain of genes such as hopanoid biosynthesis involved in membrane stabilization at low pH and the functional redundancy for generating an internal positive membrane potential revealed the transition from neutrophilic properties to a new acidophilic lifestyle by shaping the *Acidithiobacillaceae* genomic structure. The presence of a pool of accessory genes with functional redundancy provides the opportunity to “hedge bet” in rapidly changing acidic environments. Although a core of mechanisms for acid resistance was inherited vertically from an inferred neutrophilic ancestor, the majority of mechanisms, especially those potentially involved in resistance to extremely low pH were obtained from other extreme acidophiles by horizontal gene transfer (HGT) events.

Metabolite differentiation of black yeasts grown in a mars global simulant using mass spectrometry and molecular network approach

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Extremophiles are microorganisms that inhabit extreme terrestrial environments and are considered the best model of life for studies in astrobiology[1]. These microorganisms growths in extreme and hostile physicochemical conditions, such as high and low temperatures, high salinity concentration, and lack of nutrients[2]. Nowadays, it is known that the Martian surface is considered highly hostile to life. However, recent studies have shown that the Martian subsurface may contain liquid water and possibly friendly geological niches where extremophile life can be found. In this sense, to understand the microbial-mineral interaction that can occur on Mars, two species of black yeast*, Exophiala sp*. 15Lv1, isolated from the Atacama Desert, and *Exophiala oligosperma*, isolated from an acidic aqueous solution, were cultured in a Mars Global Simulant (MGS1), an assembled synthetic regolith based on mineralogical data obtained from the Curiosity mission of NASA[3]. In addition, changes in metabolite production under mineralogical conditions similar to Mars were analyzed for the first time using liquid chromatography coupled to mass spectrometry with a molecular network approach. The analysis of the extracts produced by the microorganisms using the Global Natural Product Social Molecular Networking (GNPS) platform showed differentiation in the chromatographic profile, with peaks and unique ions in the experiments where the yeast grew in the presence of the mimicked Martian regolith. This knowledge could be the beginning of understanding new metabolic pathways that was silenced, and aroused in the presence of a Mars-like chemical environment.

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Improvement of organic solvent tolerance of *Escherichia coli* by vanillin

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Organic solvents with a low log P_{OW} generally inhibit microbial growth[1]. Thus, environment containing a large amount of organic solvents is one of extreme conditions for microorganisms. Organic solvent-tolerant bacteria with internal cofactor regeneration and multistep metabolic pathways can be efficient whole-cell biocatalysts in reactions containing organic solvents. Improvement of the organic solvent tolerance of bacteria is expected to enhance the production levels of various valuable compounds such as biofuels, polymer precursors, and chiral chemicals[2].

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is used as a flavor ingredient in various food categories and is known to inhibit microbial fermentation. The vanillin is presently synthesized from various raw materials such as lignin and guaiacol. Pretreatment of plant biomass for fermentable sugar production generates vanillin as a major byproduct. Vanillin shows strong antimicrobial properties against yeast, fungi, and bacteria. In this study, we found that vanillin improved the hydrophobic organic solvent tolerance of *Escherichia coli* and showed that the AcrAB-TolC efflux pump is involved in that tolerance. The expression level of the pump was enhanced by the addition of vanillin. AcrAB-TolC efflux pump expression is known to be regulated by transcription activators such as MarA, SoxS, and Rob[3]. Among these three transcription factors, *marA* transcription was significantly increased by the addition of vanillin. We found that the AcrAB-TolC efflux pump is involved also in vanillin tolerance. The *acrB* disruptant was more sensitive to vanillin than the parent strain. A complementation test revealed that the introduction of the *acrB* gene recovered the vanillin tolerance of the *acrB* disruptant. These findings provide a new perspective on the utilization of chemicals derived from lignocellulosic biomasses and concerning the improvement of bioproduction efficiency in the presence of organic solvents.

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Thermobaculum Tajikiense sp. Nov., A novel thermophilic bacterial species and source of valuable Industrial enzymes

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There are many high-altitude geothermal springs on the territory of Tajikistan, the microbial diversity of which has not yet been studied. In an effort to recover thermophilic microorganisms and enzymes for industrial applications, a novel thermophilic bacterium belonging to the Chloroflexi phylum was isolated from hot soil (88°C; pH 7.4) in the Tamdykul geothermal region in Tajikistan, at an elevation of 2198 m. The isolate was an aerobic, non-spore-forming rod-shaped bacterium which formed pink colonies on R2A agar plates. The isolate, designated strain T2pink, grew in the temperature range from 55 – 80°C, and at pH values ranging from 5 to 10. Based on the 16S rRNA gene sequence, it was identified as a member of the *Thermobaculum* genus, sharing 94.2% sequence identity with the only described species in the is genus, *Thermobaculum terrenum*, recovered from a hot spring in the Yellowstone National Park, USA. Nanopore genome sequencing and assembly yielded two contigs, representing two chromosomes with sizes of ~2 Mb and ~1 Mb, like what is found in *Thermobaculum terrenum*. Average nucleotide identity (ANI) and digital DNA : DNA hybridization (dDDH) values as compared to the *Thermobaculum terenum* genome were 89.5% and 36.5%, respectively, demonstrating that strain T2pink constitutes a separate and distinct genome species, and the first reported thermophile from Tajikistan. Further genome similarities are discussed. The dominant cellular fatty acids of strain T2pink were 18:0 (24.4%), 17:0 iso (12.6%), 19:0 iso (14.6%); 19:0 anteiso (12.6%); 20:0 (17.2%) and 19:0 (8.2%). Strain T2pink actively degraded cellulose, casein, starch, and amylase, and yielded positive results for a large number of hydrolytic enzymes e.g., C8 esterase lipase, lipase (C), amino acid arylamidases, β-galactosidase, glucuronidase, α-glucosidase, α-fucosidase at 65°C, and thus represents a good source of potentially valuable industrial enzymes.

Development of novel enzymatic Transglutaminase nanoflowers for biomedical applications

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The present work highlights the unique designing of the enzymatic nanoflowers made of Transglutaminase (TGase) enzyme. TGase was produced from *Streptomcyes mobaraensis*.[1] TGase being a cross-linking enzyme is the key regulator which renders microbes to thrive under hostile environmental conditions. TGase modulates the physiological functioning of the microbes by catalyzing isopeptide bond formation, thereby bestowing robust cell barriers viz., spore coat.[2] With the versatility of microbial TGase facilitating the nanoflowers formation by acting as molecular glue, it was demonstrated to have versatile properties. These enzymatic nanoflowers represent a new and elegant approach to enzyme immobilization, possessing enhanced activity, stability, durability, and even selectivity of entrapped organic biomolecules. These are endowed with diverse functionalities for bio-catalytic applications. The rapid, simple synthesis of producing immobilized enzymes acquires hierarchical nanostructures with large surface-to-volume ratio.[3] These floral molecules, owing TGase's inherent structural characteristics, were designed to have a high cargo (drug/inhibitors) loading capacity and enable effective targeted release, as reported for the first time. We envisioned TGase nanoflowers as novel nanocarriers and thus led us to explore the process for developing 3-D TGase NFs bestowed with high anti-cancer efficacy. The well-framed multi-dimensional conformation of nanoflowers tailored petal structure, conferred by enzyme TGase, is the highlight of the work. Herein, we report the anti-cancer characteristics portrayed by enzymatic TGase NFs, which are biocompatible in nature.[3] Thus, nanoflowers research is anticipated to take multiple directions for the development of drug delivery systems, biosensors, biocatalysts, bio-related devices, etc.

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Discovery and synthetic applications of novel thermostable biocatalysts from extremophiles

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Extremophiles, microorganisms naturally found in “extreme” ecological niches, produce robust enzymes for bioprocesses and product development. During the last few years, the advancement and improvement of sequence-based metagenomic techniques has allowed to circumvent the frequently observed limitations in the cultivation of extremophiles under laboratory conditions. In our labs we exploit metagenomics to discover novel (thermo)stable enzymes useful for synthetic applications. In the framework of the HOTRAM Project, a Marie Curie IEF post-doctoral programme, we discovered three novel amine transaminases (ATAs) by mining extremophilic metagenomes. In particular, one of these ATAs demonstrated to be exceptionally thermostable, with an apparent melting temperature around 88°C and retaining 85% of activity after 5 days incubation at 80°C.[1] More recently, the use of activity-guided methods, such as enrichment cultures, as an alternative and complementary discovery approach, led us to the identification of a novel thermostable β-amino acid transaminase from a *Meiothermus* strain isolated in an Icelandic hot spring showing a broad substrate scope.[2] Moreover, we discovered a library of novel hydroxysteroid dehydrogenases, enzymes so far described only from gut and soil bacteria, by mining extremophilic (meta)genomes. The results obtained in the study of the substrate promiscuity and synthetic application of these novel biocatalysts with steroidal and non-steroidal substrates will be presented.[3,4]

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Thermostable xylanases from a biogas plant microbiota

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Lignocellulose biomass is an abundant and sustainable feedstock that can be transformed into a wide variety of value-added products. However, lignocellulose valorization is challenging due to its high complexity and recalcitrance[1]. To this end, implementing thermophilic microbes and enzymes can lead to several advantages, like the increase in: i) reaction rates, ii) substrate and product solubility, and iii) diffusion coefficient[2]. In this study, a set of enrichment batches cultivations was carried out to isolate microorganisms producing lignocellulolytic enzymes from a local biogas plant, fed with livestock effluents and agricultural by-products. Enrichment cultivations of microbial consortia were run for ten days, using the wheat straw litter (from edible mushroom production) as the sole carbon source. Cultivations were carried out at 37°C, 50°C, and 70°C to favor the proliferation of both mesophilic and thermophilic microbes. Moreover, the consortia were sub-enriched on agar plates containing carboxymethyl cellulose (CMC) or xylan as exclusive carbon sources. To induce enzyme production and secretion, the sub-enriched consortia were cultivated in flasks containing sterile mushroom litter and digestate (from the biogas plant). We daily assessed microbial growth as well as the activity of endo-β-1,4-glucanase and endo-1,4-β-xylanase via Azo-CMC and Azo-xylan degradation assay, respectively. The sub-enriched consortia at 37°C and 50°C were found to secrete robust xylanases, whereas no Azo-CMC degradation was detected. Unconcentrated cell-free supernatants from batches cultivated at 37°C were shown to be active over a pH range of 4 to 10, displaying their optimum at pH 7. Xylanases activity was highest at 60°C, although at 50°C the enzymes preserved more than 80% of their maximal activity up to three days of incubation. In addition, the supernatants are currently being challenged against pretreated mushroom litter, coffee spent grounds[3], and pure xylan to evaluate their activity towards industrial-like biomasses. Finally, the enriched consortia at 50°C and 70°C are currently being characterized with positive preliminary results.

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Impact of human activity on the presence of antibiotic resistance in cryospheric microbial communities in the Alpine space

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In recent years, antibiotic (AB)-resistances are attracting more and more attention but are still investigated mainly in urbanized areas. Only a few studies focus on the presence of (mutli-)resistant microorganisms in natural (extreme) habitats. This work provides a detailed report about the current situation of AB-resistance in cryospheric habitats in the Tyrolean alpine space and the impact of anthropogenic influence on the rise of resistant psychrotolerant and psychrophilic bacteria in said regions.

To evaluate antibiotic susceptibility of isolated cold-loving microorganisms from three different glaciers and their melting waters, an optimized protocol of the agar-disk-diffusion method was used to test a total of 266 bacterial isolates from snow, ice and freshwaters on their ability to withstand eight different antibiotics. With this method, 72 % of all isolates could be identified as multi-resistant whereas only 18 % were susceptible to every antibiotic tested.

To evaluate the effect of the anthropogenic influence, which is one of the main factors known for the emergence of antibiotic resistance, we combined the data from our antibiogram with different data that indicates human impact (e.g. tracking data, level of urbanization, sewage water treatment plants). This way we could show that the direct human impact is having a significant effect on the presence of antibiotic resistance even in cryospheric and seemingly less impacted habitats by humans. However, even remote environments inhabit AB-resistant bacteria hence highlighting that further factors like intrinsic resistance mechanisms and global distribution via different vehicles must be taken into account to get a better understanding of antibiotic resistance in cold habitats.

Molecular mechanisms behind protein halotolerance

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Proteins of halophilic organisms that accumulate molar concentrations of KCl in their cytoplasm have much higher content in acidic amino acids than proteins of normal organisms. It has been proposed that this excess is necessary to maintain proteins hydrated in an environment with low water activity: either via direct interactions between water and the carboxylate groups of acidic amino acids or via cooperative interactions between acidic amino acids and hydrated cations, which would stabilize the folded protein. Our simulation study of 5 halophilic proteins and 5 mesophilic counterparts does not support either possibility. The simulations use the AMBER ff14SB force field, with newly optimized Lennard-Jones parameters for the interactions between carboxylate groups and potassium ions. We find that proteins with a larger fraction of acidic amino acids indeed have higher hydration levels, as measured by the concentration of water in their hydration shell and the number of water-to-protein hydrogen bonds. However, the hydration level of each protein is identical at low (b_{KCl} = 0.15 mol/kg) and high (b_{KCl} = 2 mol/kg) KCl concentration: excess acidic amino acids is clearly not necessary to maintain proteins hydrated at high salt concentration. It has also been proposed that cooperative interactions between acidic amino acids in halophilic proteins and hydrated cations stabilize the folded protein structure, and would lead to slower dynamics of the solvation shell. We find that the translational dynamics of the solvation shell is barely distinguishable between halophilic and mesophilic proteins; if such a cooperative effect exists, it does not have that entropic signature. We want to further validate our conclusions, using experiment. We have made a collaboration with multiple groups, so we can characterize protein solvation as a function of [KCl] concentration using IR solvation shell spectroscopy experiments. The experiments are not finished, but if available, we intend to present it at the conference.

Acid whey as a prospective substrate for biomass production of thermostable α-amylase producing recombinant *e. Coli* bl21 strain

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Acid whey is a highly valuable waste and could have important applications in biotechnology, especially in recombinant enzyme industry[1]. The goal of the current work was the optimization of acid whey for the production of thermostable ***α***-amylase originated from *Anoxybacillus karvacharensis* K1 strain (=DSM 106524^T=KCTC 15807^T). The gene was cloned in pET-21b transcription vector and expressed in *Escherichia coli* BL21 cells. The pH of acid whey was adjusted to 7.0 by NaOH, then sterilized by autoclaving (105 ^{o}C, 20 min). The ability of recombinant *E. coli* BL21 strain to grow in acid whey was studied and compared with commercial Nutrient Broth (NB) media. The growth conditions in both media were 37^{o}C and 150 rpm. The *α*-amylase in the lysed cells, obtained from both culture media, was purified using Profinity IMAC Ni-charged resin. The obtained fractions were visualized using SDS-PAGE. Sugar quantity was measured by 3,5 dinitrosalicylic acid method[2]. The optical density of the bacterial suspension in acid whey and NB media was reached 2.77 and 1.26, respectively after 72 hours of incubation. The recombinant ***α***-amylase protein had around 70 kDa molecular weight, which has maximal activity at pH 7.0-7.6 and 60-65 ^{o}C. The relative activity of *α*-amylase was 2.92 times more in case of acid whey (0.193 U/ml) than in NB (0.066U/ml). The highest activity was observed in the third fraction of the protein purification by His B buffer (50mM KH_2PO_4, 300mM NaCl, 500mM imidazole), which was 3.41 U/ml.

Acknowledgment: This work was supported by the Foundation of Armenian Science and Technology (FAST) ADVANCE program.

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Geological controls on the deep subsurface biosphere

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Despite being one of the largest microbial ecosystems on Earth, with >10^{29} microbial cells, many basic open questions remain about how life exists and thrives in the deep subsurface[1]. Much of this ambiguity is due to the fact that it is exceedingly difficult and (often prohibitively expensive) to sample the deep subsurface biosphere, requiring elaborate drilling programs and/or access to deep mines. We propose a new sampling approach which involves collection of a large suite of geological, geochemical, and biological data from many deeply-sourced seeps over large spatial scales[2]. This enables research into interactions between the geosphere and the biosphere, expanding the classic local approach to regional or even planetary scales. Understanding the interplay between geology and biology on such scales is essential for building models of the subsurface ecosystem and extrapolating ecological roles of subsurface microbes beyond single sample interpretations and laboratory experiments. This approach has been used successfully across the Central American and South American Convergent Margins[2,3,4], and can be applied more broadly to other types of geological regions (i.e., actively rifting, intraplate volcanic and/or hydrothermal settings), providing access to a framework for interpreting evolution and ecosystem functions through deep time.

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Training the next generation of extreme researchers: innovative master degree in the biology of extreme environments

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Extreme environments play a key role in regulating global biogeochemical cycle, harbour an enormous diversity of microbial organisms and provide unique biotechnological solutions to our society[1]. Additionally they are unique ecosystems that can be used as proxy to understand the evolution of life on our planet and provide analogs environments for planetary and astrobiological investigations[2]. The study the biology of extreme environments requires specific skills, and a diverse background that lays at the interface between diverse disciplines, such as biology, chemistry, geochemistry, geology and astrophysics to mention a few. Despite this, current curricula that focus on the biology of extreme environments are scarce in our educational systems, and specific courses are few and far between. At the University of Naples we have designed a unique Master Degree program specializing in the Biology of Extreme Environments. The program is designed around two curricula: one focusing on the Biological Resources of extreme environments, looking primarily at the possible biotechnological applications of extremophiles; the other on Astrobiology, focusing on the origin of life and its search in the universe. The program is designed to be highly interdisciplinary, providing hands on practical experience in the diverse disciplines of the programs and aims at preparing the next generation of researchers in the field of extremophiles and extreme environments.

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Diversity of psychrophilic methanotrophs from the barents sea seabed revealed by enrichments and reconstruction of metagenome-assembled genomes

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Methane-oxidizing bacteria (MOB), also known as methanotrophs, play an important role in the global methane cycle due to their ability to act as a natural methane sink reducing the release of methane to the atmosphere. Methanotrophs have been recovered from a large number of environments, but isolates from cold deep-sea environments are scarce, especially within the 'deep sea-1' clade, with only one cultivated species, *Methyloprofundus sedimenti*[1]. Molecular techniques have been extensively implemented to study the diversity of these microorganisms mostly by targeting the genes 16S rRNA and *pmo*A, that encodes the alpha subunit of particulate methane monooxygenase. Forty-one samples of the Barents Sea seabed were collected from the top sediment layer and 5cm depth, where the in situ temperature varied from 0.1 to 4°C, and water depth from 0.5 to 1 km. Enrichments and multiple cultural dilutions of the seabed samples were prepared with methane as the only carbon and energy source, and were incubated under aerobic conditions at 10°C. Illumina shot-gun metagenomics analysis was used to assess the microbial diversity of 21 positive methane-oxidizing cultures. Assembly and binning yielded 12 Metagenome-Assembled Genomes (MAGs) classified as methanotrophs with sizes that varied between 3.1Mb to 4.2Mb and estimated completeness ranging from 75.9 to 99.3%. All the MAGs represented type I methanotrophs (Gammaproteobacteria), and were tentatively assigned to genus *Methyloprofundus* (family *Methylococcaceae*) with closest placement ANI values ranging from 82.5 to 93.1% and GC values ranging from 40.4 to 41.4%. The functional gene *pmo*A was found in all the MAGs and were affiliated with the *Methyloprofundus* genus and uncultivated methanotrophic endosymbionts of *Bathymodiolus* mussels. It can be concluded that the diversity of the psychrophilic methanotrophic communities in the seabed of The Barents Sea is rather limited, and dominated by novel species belonging to the family *Methylococcaceae*.

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Novel esterases from thermal vents of Ischia Island (Italy)

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Naïve screening-based mining of metagenomic libraries is one of the central approaches for discovery of novel enzymes for a range of applications. Extremophilic microorganisms and their communities are of a particular interest as a source of enzymes that may be readily applicable under conditions of industrial processes that often operate at elevated temperatures and pressure and in presence of organic solvents. In this study, we used samples taken in September 2018 in hot springs of Ischia Island (Italy) in Cavascura (45-55 ºC, pH 8) and Maronti Beach (75-90 ºC, pH 4.5), spiked them with the three different bio-based polyester plastic materials and incubated at *in situ* temperatures. Microbial diversity was assessed using Illumina MiSeq (V4) 16S rRNA gene amplicon sequencing. Simultaneously, DNA was used to produce fosmid libraries in *E. coli* pCC2FOS vector. The libraries were arrayed in 384-well multititre plates and subjected to colony-based screening using general assays for lipases/esterases and other hydrolases (PMID: 26266751) at 37, 50 and 70º C. Approx. 60 hits were obtained after screening of 7000 clones, with a third detected only at 50 °C and higher. Positive fosmids were sequenced, genes of interest were identified and cloned into a pET-46 Ek/LIC plasmids, expressed in *E. coli*, proteins were purified and assayed using standard and industrially-relevant substrates. Three of carboxylesterases, apparently from the yet uncultured *Chloroflexota*, exhibited temperature optima (50, 60 and 70-80ºC). They demonstrated an elevated substrate promiscuity: of 44 structurally distinct ester substrates 25-44 were hydrolysed, in addition to the hydrolysis of polylactic acid (PLA) and trimeric polyethylene terephthalate (3PET). The crystal structure of one of these enzymes, which showed the best thermostability (retaining 80-90% activity after 120 h at 80ºC) revealed the presence of the N-terminal β-lactamase-like domain and C-terminal lipocalin domain with the catalytic site cleft with catalytic residues S68-K71, Y160-N162 and H299, as well as several hydrophobic and polar residues essential for substrate binding. The presentation will provide further information on characterized enzymes and address the thermophilic community composition changes upon incubation with polyester plastics in more detail.

Extracellular enzyme activity by soil thermophiles. An example of adaptation to temperature and desiccation

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Microorganisms are critical for the functioning of soil biogeochemical cycles. Microbial decomposition of soil organic matter is limited by the activity of the extracellular enzymes by microorganisms. Moreover, soil upper layers are exposed to drastic changes in temperature and dryness throughout daily and seasonal cycles. Thermophiles have been reported as ubiquotous in soils although their role in temperate environments remains poorly understood[1]. Herein, we aim to study the capability of soil extracellular enzymes from these organisms to cope with extreme conditions observed in soils, such as high temperature and desiccation. Results indicate that soils frequently exposed to high temperatures and dryness (e.g., Southern Spain) present optimum extracellular enzyme activities at high temperature (60ºC-75ºC) and reduced water content (0.4-0.7 water activity). However, extracellular enzymes at soils that are rarely exposed to high temperatures (Northern Spain), and show high humidity, presented optimum activities at high temperature (55ºC-70ºC) but only under high water content (0.9-1 water activity). Similar measurements with soil isolates showed that, for example, isolates of the soil thermophile Parageobacillus thermoglucosidasius present optimum activities at high temperatures (60ºC-75ºC) and low water content (0.4-0.7 water activity) [2]. Consequently, some soil thermophiles present adaptations to the conditions of high temperature and desiccation observed in soil upper layers. In contrast, mesophilic strains (i.e., Pseudomonas) presented optimum extracellular activities at lower temperature and high water activity (0.9-1). These differences confirm a distribution of performance as a function of environmental conditions and a separation of resources at the microbial scale which is essential for to understand bacterial growth, the processing of soil organic matter, the actual relevance of microorganisms in the biogeochemical cycles and their potential effects on a global scale in relationship to climate warming.

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Metagenomic responses of eastern mediterranean deep-water microbial communities to hydrocarbon contamination at in situ pressure

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Microbial organic matter degradation is pressure-sensitive and negatively impacted by decompression of deep-sea water samples [1]. Furthermore, removing hydrostatic pressure will likely alter the overall sign and strength of interspecies interactions with subsequent consequences on the microbial community structure and function [2,3]. Following the Deepwater Horizon (DWH) accident in the Gulf of Mexico, the contamination of deep-sea ecosystems with hydrocarbons and the crucial role of indigenous microbes in bioremediation was acknowledged. Yet, the study of deep-sea oil biodegradation is largely carried out using decompressed microbial communities. Here, we emulated a deep oil plume in the Eastern Mediterranean Sea (EMS), similar to that observed in DWH, using un-decompressed seawater and followed the taxonomic and functional response of the mesopelagic microbial community to oil contamination and dispersant application for 77 days at *in situ* pressure (10Mpa) and temperature (14^{o}C). Our results show that the obligate hydrocarbon-degrading bacteria (OHCB) *Alcanivorax* and *Marinobacter* play a major role in deep-sea bioremediation in the EMS, contrary to observations so far that high hydrostatic pressure selectively inhibits OHCB in favour of non-specific oil-degrading taxa [4]. Gene functions and metabolic pathways that became enriched during hydrocarbon degradation before and after dispersant application were analysed by shotgun metagenomic sequencing. This study demonstrates the importance of studying microbial function under realistic pressure conditions for the development of oil bioremediation protocols in the deep-sea.

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Enrichment and isolation of piezotolerant hydrocarbon degraders from the deep waters of the Eastern Mediterranean Sea

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Deep sea microbial degradation of hydrocarbons has been thoroughly studied in the Gulf of Mexico after the Deepwater Horizon (DWH) accident in 2010 [[1]]. However, the vast majority of research has been conducted with decompressed microbial communities which were incubated either at atmospheric pressure (P) or re-pressurized in the lab [[2,3]]. Disruption of *in situ* P alters the community diversity and thus affects the overall metabolic capability including hydrocarbon degradation rates [[4, 5]]. In this study, un-decompressed and decompressed microbial communities were collected from the deep waters of the Eastern Mediterranean Sea (EMS) and were enriched in hydrocarbon degraders, in the presence of oil and dispersed oil at *in situ* P and temperature, in order to address the effect of decompression and dispersant on oil removal rates and microbial structure. Bacterial strains were isolated from each treatment and were subjected in synergistic experiments under atmospheric and high pressure to assess any possible increase in degradation rates. Since oil and gas exploration activities in the EMS are intensifying and moving towards deeper and more challenging waters, the results from this study aim to elucidate the importance of pressure when experimenting with deep water communities and provide insight on important strains that might be used in bioaugmentation protocols for bioremediation in the EMS.

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Relationships of molecular tunnels in enzymes of methanogenic Archaea and their growth temperature

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Recent analyses of protein structure and the existence of molecular channels in enzymes of thermophiles have suggested a critical role of spatial voids in proteins, above all, those enzymes functioning under high temperature[1]. These spaces within the protein structure are required to access the active site and to maximize availability and thermal stability of their substrates and cofactors. Methanogens are a singular phylogenetic group of Archaea which perform anaerobic respiration producing methane during growth. Methanogens inhabit a variety of environments including the full range of temperature for the known living forms. Herein, we carry out a dimensional analysis of molecular tunnels in key enzymes of the methanogenic pathway from methanogenic archaea growing over a broad temperature range. We aim to determine whether the dimensions of the molecular tunnels are critical for those enzymes in relationship to growth temperature. Results showed that as increasing growth temperature the dimensions of molecular tunnels in the enzymes formylmethanofuran dehydrogenase, methyl-CoM reductase and heterodisulfide reductase narrows down to highly strict limits at the highest growth temperature, i.e., for hyperthermophilic methanogens. However, growth at lower temperature permits a wide dimensional range for the molecular spaces in these enzymes. This is in agreement to previous suggestions on a major role of molecular tunnels for the activity of some enzymes under high temperature growth[1, 2]. These results contribute to better understand archaeal growth at high temperatures, and they indicate that the optimization of restrictive dimensions of molecular tunnels at increasing growth temperatures represent an important adaptation that allows Archaea to thrive under high temperature conditions.

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Potential horizontal gene transfer events detected from the genomes of *Parageobacillus Thermoglucosidasius*

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The pan-genome includes the complete pool of genes of a species including those recently acquired. These new additions of genetic material are frequently linked to horizontal gene transfer (HGT) processes[1], and can confer adaptive advantages improving their functional response and growth. Previous studies have reported that *Parageobacillus* frequently exchanges DNA with other Firmicutes sharing similar environments[2]. In this work, based on the pan-genome of *Parageobacillus thermoglucosidasius,* we selected some genes rarely observed in this species to infer potential HGT events. The Blast algorithm was used to find similar genes within the phylum and among other more distant bacteria. Results confirm frequent DNA exchange with other Firmicutes. These analyses also revealed genetic exchanges with other phyla, such as Actinobacteria, Proteobacteria and Bacteroidetes-Chlorobi. Among these cases, operons annotated as ABC (ATP binding cassette) transporters are frequently detected. The frequency of highly similar ABC transportes detected in those bacterial taxa suggested a directional HGT with origin within the phylum Firmicutes. This exploratory analysis indicates that Firmicutes are able to export DNA to phylogenetically distant groups besides highly active DNA transfer within the phylum. These long-distance HGT can assist to better understand evolutionary HGT processes among procaryotes.

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Deinococcus species as a tool for bioremediation of heavy-metals

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Extremophiles are a rich unexploited reservoir of genetic and metabolic diversity with the ability to withstand a myriad of harsh environmental condition. *Deinococcus* genus, encompass radiation resistance bacteria such as *Deinoccocus radiodurans*, a polyextremophile resistant to UV radiation, desiccation, ionizing radiation, oxidative stress[1,2]. Moreover, *Deinococcus indicus*, is able to withstand high radiation doses and also present resistance to heavy-metals such as arsenate[3].
Over the last years, we have been study *D. radiodurans* on its molecular mechanisms associated with the resistance after a stress stimulus, namely radiation or oxidative stress. We have been focus our studies on the DNA-binding proteins which are nano-cage proteins; and the cellular homeostasis of different chemical elements, namely manganese and phosphorous[4-7]. To guarantee a sustainable practice, we are now exploring these two radiation resistant bacteria as a bioremediation system to detoxify heavy-metals from wastewater systems. Currently, we aim to address the applicability of *D. radiodurans* Dps nano-cage protein (Dps) has a tool to detoxify heavy-metals while at the cellular level we are exploiting *D. indicus* ability to resist arsenic be used has a platform for arsenate bioremediation.

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***Funding acknowledgement*s:**

- INSTRUCT-ERIC-Access Proposal, PID: 12696- „'Unveiling the molecular details of the Dps-DNA complex interaction, using Single particle analysis", Electron Microscopy Unit, University of Helsinki, Finland
- Fundação para a Ciência e Tecnologia, Project- PTDC/BIA-BQM/31317/2017, Metals in the radiation resistance bacterium *Deinococcus radiodurans*, its role in protection up to future applications.
- **European Union's Horizon 2020 research and innovation programme under grant agreement No 857203-** IMpaCT - Imaging life from Molecules to cells - building knowledge on Cryo-electron microscopy methodologies at ITQB NOVA – www.itqb.unl.pt/impact.

Effect of Mg^{2+} on cesium resistance of alkaliphilic *Microbacterium* sp. TS-1 and *Bacillus subtilis*

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Cesium (Cs) is an alkali metal, and its radioactive isotope ^{137}Cs is attracting attention as a major cause of radioactive contamination. Cs^+ is toxic to bacteria[1]. *Microbacterium* sp. TS-1 (TS-1) is an alkaliphilic bacterium isolated from a jumping spider and tolerant up to 1.2 M CsCl[2]. In previous studies, due to mutation into the magnesium transporter gene (*mgt*) in TS-1, the mutant showed Cs^+ sensitivity, but Cs^+ sensitive mutant restored Cs^+ resistance by adding $MgCl_2$ in the medium. It found the addition of Mg^{2+} to the culture medium to enhance Cs^+ resistance in common microorganisms such as *Bacillus subtilis*. This study aims to clarify the role of Mg^{2+} in the Cs^+ resistance mechanism by measuring the intracellular cation concentrations of *B. subtilis* when the Cs^+ concentration is elevated. We also aimed to investigate the effect of Cs^+ on ribosome stabilization of strains TS1 and *B. subtilis*.

The intracellular Cs^+, K^+, and Mg^{2+} concentrations of *B. subtilis* in the presence of Cs^+ showed that reducing the intracellular K^+ concentration promotes the uptake of Mg^{2+}. However, it was also clear that Mg^{2+} did not directly compensate for the decrease in K^+ concentration since the increase in intracellular Mg^{2+} concentration did not affect Cs^+ and K^+ concentrations. We investigated the effect of elevation Cs^+ on ribosome stability, indicating that the ribosomes of wild-type of strain TS-1 are stable against Cs^+. In contrast, Cs^+ sensitive mutants of TS-1 and wild type of *B. subtilis* have destroyed the 70S ribosome complex. These results suggest that Cs^+ destabilizes ribosomes and inhibits protein synthesis by decreasing K^+ concentration, but the addition of Mg^{2+} stabilizes the ribosome complex, improving the Cs^+ resistance of the microorganism.

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Alpha-amylase cazy gh families – focus on extremophiles

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The currently worldwide well-accepted database of Carbohydrate-Active enZymes – CAZy[1] – has developed from the original sequence-based classification of glycoside hydrolases (GHs)[2]. The GH part still represents the largest segment of CAZy counting 173 GH families, of which four, i.e. families GH13, GH57, GH119 and GH126, may be considered as the α-amylase GH families[3]. They have been created chronologically, i.e. GH13 in 1991[2], GH57 in 1996[4], GH119 in 2006[5] and finally GH126 in 2011[6]. While the family GH13 with more than 131 thousand members belongs to largest GH families, the family GH119 with less than 40 members may be the smallest one[1]. For the families GH13 and GH57, all main basic characteristics are known[3,7,8]: (i) structural fold adopted by the catalytic domain – a TIM-barrel for GH13 vs an incomplete TIM-barrel for GH57; (ii) catalytic machinery – Asp (nucleophile), Glu (proton donor) and Asp (transition-state stabilizer) residues in GH13, whereas Glu (nucleophile) and Asp (proton donor) residues in GH57; (iii) conserved sequence regions (CSRs) – 4-7 in GH13 and 5 in GH57; and (iv) reaction mechanism – a retaining one for both families. With regard to families GH119 and GH126, the eventual close relatedness of GH119 to GH57 has already been demonstrated[9], whereas for GH126 there is still some uncertainty with regard to its catalytic machinery[10]. α-Amylases and related amylolytic enzymes originated from extremophiles selected mainly from families GH13 and GH57 will be presented with focus on their unique sequence-structural features and remarkable evolutionary relationships.

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A new gh13 subfamily represented by amylolytic enzymes from *flavobacterium* sp. No. 92, *Bacteroides thetaiotaomicron* and *zunongwangia profunda*

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In the Carbohydrate-Active enZymes database – CAZy[1], the α-amylase family GH13 counts currently more than 136,000 sequences and ~30 different enzyme specificities. The family has already been divided into 44 subfamilies, additional subfamilies being still emerging and/or awaiting the official assignment[2,3]. The presented *in silico* study was undertaken in an effort to propose a novel GH13 subfamily represented by the three experimentally characterized amylolytic enzymes: (i) the cyclomaltodextrinase from *Flavobacterium* sp. No. 92[4]; (ii) the neopullulanase from *Bacteroides thetaiotaomicron*[5]; and (iii) the α-amylase from *Zunongwangia profunda*[6]. The searches through sequence databases resulted in collecting a convincing group of 108 homologues forming an unambiguous cluster in the evolutionary tree, well separated from representatives of all remaining GH13 subfamilies. The members of the newly proposed GH13 subfamily share a few exclusive sequence features, such as: (i) the “aromatic” end of the conserved sequence region II consisting of two well-conserved tyrosines with either glycine, serine or proline in the middle; and (ii) a glutamic acid that succeeds the catalytic proton donor in the conserved sequence region III. Concerning the domain N present in the three experimentally characterized members of the new GH13 subfamily, it has been hypothesized – based on docking trials of this domain from the cyclomaltodextrinase from *Flavobacterium* sp. No. 92 with α-, β- and γ-cyclodextrins – it may represent a new type of a starch-binding domain.

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Fervidobacterium pennivorans subspecies *keratinolyticus*, a feather-degrading anaerobic thermophile

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A new thermophilic bacterium termed *Fervidobacterium pennivorans* subspecies *keratinolyticus* was isolated from a high-altitude terrestrial hot spring in Tajikistan. This strain is a strictly anaerobic rod, with cells occurring singly, in pairs or as short chains, with a generation time of 150 minutes. It grows well at 70 °C and neutral pH. Peptone, glucose and galactose are its preferred substates. Its genome has a total size near 2 Mb, with a DNA G+C content of 39.0%. Genomic comparison showed an 81% overall in-silico nucleotide sequence identity with the *Fervidobacterium pennivorans* type strain, DSM9078. Compared to the type strain, subspecies *keratinolyticus* possesses a large (1.112.708 bp) chromosomal inversion, identified both by Pacbio and Nanopore genome sequencing. Subspecies *keratinolyticus* is capable to grow on chicken feathers at 70 °C, breaking down the feather keratin very efficiently. The keratinolytic activity is an unusual feature shared with other members of genus *Fervidobacterium*. This work presents this new keratinolytic strain, its full genome sequence and comparison with the other members of genus *Fervidobacterium*.

A key role of energy metabolism regulation in adaptation to high hydrostatic pressure in the hyperthermophilic piezophilic archaeon *Thermococcus barophilus*

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Thermococcus barophilus, a piezophilic hyperthermophilic archaea is used as a model to characterise the mechanisms involved in high hydrostatic pressure (HHP) adaptation. Our findings shown that the expression of a number of gene clusters is modulated by hydrostatic pressure, including those of energy metabolism[1]. In Thermococcales, all the energy metabolism genes are under the influence of the SurR regulator[2]. To gain further insight into how HHP regulates the expression of genes related to energy metabolism, several gene deletion mutants were constructed, including *surR*. Then, their roles were assessed through several culture configurations including different pressures (0.1 MPa, 40 MPa and 70 MPa) in the absence and presence of sulfur.
The phenotype analysis of the *surR* mutant revealed the effect of SurR on the growth and expression of targeted genes, regardless of sulfur content, which is distinct from the results observed in other non-piezophilic Thermococcales species (e.g. *Pyrococcus furiosus* and *Thermococcus kodakarensis*)[2,3]. These results suggest that the physiological role/state of this regulator in *T. barophilus* is impacted by hydrostatic pressure.

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Identification of enzymes involved in lipoyl-protein biosynthesis in the hyperthermophilic archaeon *thermococcus kodakarensis*

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Lipoate is a sulfur-containing cofactor and a component of the glycine cleavage system (GCS) involved in one-carbon metabolism such as serine generation from glycine. In the bacterium *Esche-richia coli*, lipoyl synthase *Ec*-LipA catalyzes the insertion of two sulfur atoms to the C6 and C8 positions of the octanoyl moiety on the octanoyl-H-protein to produce lipoyl-H-protein. Another protein lipoate-protein ligase (Lpl) *Ec*-LplA, an ATP-dependent enzyme, can salvage and attach the free lipoate or octanoate to the H-protein (Fig. 1). Although the hyperthermophilic archaeon *Thermococcus kodakarensis* can synthesize lipoyl-H-protein, a gene homologous to the classical lipoyl synthase, *Ec*-LipA, cannot be found on its genome. Genome information suggested that the TK2109 and TK2248 genes in *T. kodakarensis* are involved in lipoyl-H-protein biosynthesis. Gene disruptions of the two genes resulted in lipoate/serine auxotrophy and their purified recombinant proteins exhibited lipoyl synthase activity. The results indicated that the TK2109 and TK2248 genes encode a structurally novel lipoyl synthase (*Tk*-LipS) [1]. In addition to the *de novo* lipoyl-protein biosynthesis enzyme, *T. kodakarensis* possesses a putative Lpl encoded by TK1234 and TK1908. Their recombinant proteins together displayed significant ligase activity toward octanoate and lipoate. Although Lpl has been considered responsible for lipoate salvage, gene disruption of TK1908 led to lipoate/serine auxotrophy. The results implied that, in addition to lipoate salvage, these proteins function in *de novo* lipoyl-protein biosynthesis through their octanoate-protein ligase activity.

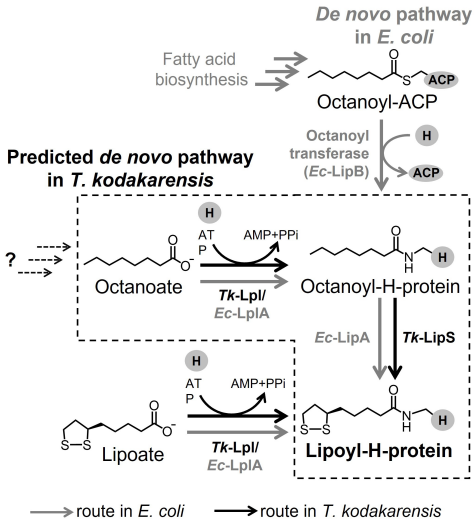

Fig. 1. H-protein lipoylation in *E. coli* and *T. kodakarensis*.

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Dredging and deposition of metal sulfide rich river sediments results in rapid conversion to acid sulfate soil materials

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Sediments along the Baltic Sea coast can contain considerable amounts of metal sulfides that if dredged and the spoils deposited such that they are exposed to air, can release high concentrations of acid and toxic metals into recipient water bodies. Two river estuaries in western Finland were dredged from 2013 to 2014 and the dredge spoils were deposited on land previously covered with lime to buffer the pH and mitigate acid and metal release. In this study, the geochemistry and 16S rRNA gene amplicon based microbial communities were investigated to explore whether the application of lime prevented a conversion of the dredge spoils into acid producing and metal releasing soil. The pH of the dredge spoils decreased with lime indicating metal sulfide oxidation and resulted in elevated sulfate concentrations along with a concomitant release of metals. However, calculations indicated only approximately 5% of the added lime had been dissolved. The microbial communities decreased in diversity with the lowering of the pH as taxa most similar to extremely acidophilic sulfur, and in some cases iron oxidizing Acidithiobacillus species became the dominant characterized genus in the deposited dredge spoils as the oxidation front moved deeper. In addition, other taxa characterized as involved in oxidation of iron and sulfur were identified including Gallionella, Metallibacterium, and Sulfuricurvum. These data suggested there was a rapid conversion of the spoils to severely acidic soil similar to actual acid sulfate soil and that the lime placed on the land prior to deposition, and later ploughed into the dry spoils, was insufficient to halt this process. Hence, future deposition of dredge spoils containing metal sulfides should not only take into account the amount of lime used for buffering but also its grain size and mixing into the soil.

Atypical sulfur assimilation and Cys synthesis in *Hydrogenobacter thermophilus*

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Many microbes assimilate sulfate and thiosulfate as a sulfur donor, synthesizing Cys as a key sulfur-containing metabolite. In many bacteria, O-acetylserine (acetyl-Ser) and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) are known to serve as essential precursors in this metabolism.
Hydrogenobacter thermophilus, a thermophilic hydrogen-oxidizing autotroph, belongs to the phylum *Aquificota*, the deepest branch of the domain Bacteria. With this distinctive phylogenetic position as a backdrop, many unique and novel features have been found in metabolism of this bacterium, including carbon anabolism[1,2], nitrogen anabolism[3], and syntheses of amino acids[4-6].
Here we clarify the sulfur anabolism in *H. thermophilus*. Interestingly, the Cys synthesis pathway in this bacterium involves neither acetyl-Ser nor PAPS, consisting of fewer numbers of intermediates and enzymes than those of known bacteria. The concise structure of the sulfur assimilatory pathway in *H. thermophilus* suggests that such simple pathway may be an ancestral type and that the well-known pathway might have evolved from this by acquiring new reaction steps with unstable intermediates.

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Identification of a potential second chitin assimilation pathway in the Hyperthermophilic Archaeon, *Pyrococcus Chitonophagus*

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Chitin is a natural polysaccharide composed of *N*-acetyl-D-glucosamine (GlcNAc) linked linearly with β-1,4 glycosidic linkages, and is the second most abundant biomass after cellulose. *Pyrococcus chitonophagus* (previously referred to as *Thermococcus chitonophagus*) identified by the group of Dr. R. Huber[1], is a hyperthermophilic archaeon that can degrade and assimilate chitin. Previously, our group has identified the complete chitin degradation and assimilation pathway in the closely related *Thermococcus kodakarensis*[2-4]. The genome analysis of *P. chitonophagus*[5] revealed that this archaeon harbors a complete set of genes homologous to those of *T. kodakarensis*. *P. chitonophagus* additionally contains two more chitinase genes (ChiC and ChiD[6]) other than ChiA found in *T. kodakarensis*, suggesting a greater capacity of *P. chitonophagus* for chitin degradation and assimilation. In the chitin degradation pathway of *T. kodakarensis*, GlcNAc is first deacetylated, followed by phosphorylation to form glucosamine 6-phosphate (GlcN6P). Interestingly, *P. chitonophagus* possesses other genes, CHITON_0804 [GlcNAc 6-phosphate deacetylase] and CHITON_0815 (Kinase similar to eukaryotic-like GlcNAc kinase) that are potentially involved in chitin degradation. Recombinant proteins were produced in *Escherichia coli*, and the purified enzymes displayed the predicted activities, suggesting that this archaeon has a second pathway for the conversion of GlcNAc to GlcN6P, in which the phosphorylation reaction precedes the deacetylation reaction.

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Improvement of stress tolerance of *Escherichia coli* by a novel Hydrophobic proline rich oligopeptide from *deinococcus radiodurans*

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PprI is present only in *Deinococcus* species and is known to be involved in the regulation of the expression of DNA repair genes[1]. It has been reported in several papers that *Escherichia coli* expressing the *Deinococcus radiodurans pprI* gene is resistant to various stresses such as heat and hydrogen peroxide[2-7]. However, the reinvestigation of the expression plasmid construction in the previous studies led us to hypothesize that an oligopeptide consisting of 14 residues rich in hydrophobic amino acids and proline, but not PprI, is involved in the enhanced tolerance of *E. coli*. In this study, we examined *E. coli* stress tolerance expressing *D. radiodurans* DNA fragments including the *pprI* gene to verify our hypothesis.

The expression level of gene products was investigated using in the luciferase reporter assay. As a result, the expression level of the novel hydrophobic proline rich oligopeptide was found to be 22-fold higher than that of the oligopeptide consisted of N-terminal region of PprI when the expression was under the control of the *E. coli lac* promoter. The reading frame for the former oligopeptide is displaced by –2 bases compared to that of the latter oligopeptide. After heat (52°C, 1 h) and hydrogen peroxide (0.1%, 10 min) treatment, *E. coli* expressing the novel hydrophobic proline rich oligopeptide exhibited more tolerance than the control. The enhanced tolerance was also observed in *E. coli* without entire *pprI* gene. This result was further confirmed by Western blotting analysis. The amount of PprI was not correlated with the level of stress tolerance in *E. coli*.

Our results strongly suggest that the enhanced tolerance of *E. coli* is due to the novel hydrophobic proline rich oligopeptide whose reading frame is displaced by –2 bases compared to that of PprI. We designated the novel hydrophobic proline rich oligopeptide HyPOP.

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Distinct lineages of Thaumarchaeota control nitrification in arctic environments

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Ammonia oxidizing archaea (AOA) of the class Nitrososphaeria are key players in the global nitrogen cycle, one of the most perturbed biogeochemical cycles due to anthropogenic interference. Fragile arctic ecosystems are especially affected as a result of rising temperatures accelerating permafrost thawing, and potentially leading to the release of gigatons of stored carbon and nitrogen in the soil, in the form of the greenhouse gases methane (CH_4) and nitrous oxide (N_2O)[1]. In these environments, AOA are the only detectable nitrifiers in the absence of ammonia oxidizing bacteria or comammox[2,3]. Previous meta-analyses enabled by an abundance of sequencing data have indicated that AOA are not functionally homogeneous, revealing distinct ecophysiological patterns among AOA lineages[4]. These are corroborated by physiological investigations of representative isolates of various lineages. Intriguingly, two distinct lineages of AOA, NS-ζ and NS-γ have been shown to dominate nitrifier communities in isolated analyses of arctic ecosystems[2,5]. In this study, in order to evaluate the generalized application of these observations, we systematically investigate the diversity of AOA in topsoil samples from four circumpolar tundra sites from latitudes >70°N. Our results offer new light on the distinct composition of microbial communities in arctic ecosystems with a focus on nitrifiers, raising questions about their psychrotolerant adaptations and unique metabolic capabilities.

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Natural transformation and type IV pilus biogenesis in *Thermus thermophilus*: DNA binding and interaction of pilins with the inner membrane platform

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Natural transformation, the bacterial uptake and incorporation of naked DNA, is a major force for bacterial adaption to changing environments and natural transformation. To gain insights into structure and function of natural transformation systems we have chosen the highly transformable thermophilic bacterium *Thermus thermophilus* as model organism. In *T. thermophilus* DNA uptake is functionally linked to type IV pili and mediated by a machinery composed of at least 16 subunits such as an outer membrane channel formed by the secretin PilQ, four pilin subunits (PilA1 - A4) suggested to form a DNA transporter pseudopilus and an inner membrane (IM) assembly platform comprising of PilM, PilN and PilO [1]. The latter interacts with the assembly ATPase PilF [2].
A gene cluster encoding the three minor pilins PilA1, PilA2 and PilA3 is essential for natural transformation but not for pilus formation whereas the major pilin PilA4 plays a dual role in both. Generation of single pilin mutants followed by mutant studies revealed that each of the minor pilins is essential for natural transformation [3]. Two of them, PilA1 and PilA2 bind dsDNA. PilA1 and PilA3 are both present in the IM but are absent in the outer membrane (OM), whereas PilA2 is present in both membranes. All three minor pilins, in contrast to PilA4, were not detected in long T4P structures. Moreover, PilA1 was found to interact with PilM and PilO subunits of the IM assembly platform.
Taken together we propose that a DNA translocator pseudopilus comprising of PilA1 - A4 extends through the periplasm into the secretin channel in the OM thereby binding the incoming DNA and contributing to its transport through the OM. Whether the DNA is pulled through the OM by depolimerization of the pseudopilus is one of the major question for future studies.

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Enhancing the thermostability of ppra, the dna repair protein in *deinococcus radiodurans*

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Deinococcus radiodurans is one of the strongest radiation-resistant bacteria, and it has been listed as the world's toughest known bacterium in the Guinness Book of World Records. Its extraordinary radiation resistance is due to a *Deinococcus* spp. specific non-homologous end-joining DNA repair mechanism[1]. In this mechanism, we are focusing on PprA, which is a *Deinococcus* specific protein that preferentially binds to DNA double strand breaks (DSBs)[2]. PprA is also known to play a role in shielding DNA DSBs from exonucleases and subsequent degradation. It is also able to stimulate the ligation activity, of not only *D. radiodurans* own DNA ligase, but also T4 DNA ligase and *E. coli* DNA ligase. Because of this universal ability, PprA has potential as a reagent in DNA engineering, and it is already commercialized as part of the TA-blunt ligation kit (Nippon Gene Co. Ltd., Japan). To improve the stability of the protein and characterize its effects on thermostable DNA repair proteins, such as DNA polymerase for PCR, we aim to enhance PprA thermostability. First, site-directed mutagenesis was performed to construct single amino acid variants (SAVs): eight proline-residue-, two hydrophobic-bond-, four salt-bridge- and one hydrogen-bond-introduced SAVs. Results of the heterologous expression using the pET system in *E. coli* BL21(DE3) showed that four of the 15 SAVs disrupted the protein structure and located in the insoluble fraction. The remaining 11 SAVs were purified using metal affinity resin and heat treatment was performed at 90°C for 10–60 min. The DNA binding ability was confirmed by electrophoretic mobility shift assay (EMSA) using *Eco*RI-treated linear pUC19. As results, eight SAVs had increased thermostability when compared to the WT. Currently, we are constructing multiple amino acid variants, confirming their thermostability (Fig. 1) and we will examine their effects on DNA engineering.

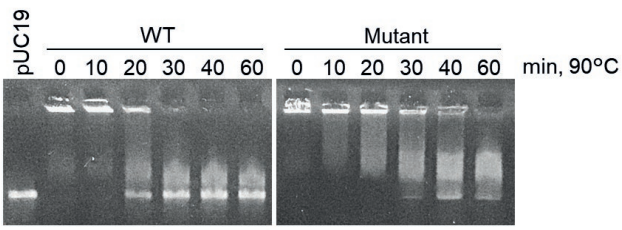

Fig. 1. EMSA of the binding of PprA to DNA. Reaction mixture containing 2 ng/µl pUC19 linearized with *Eco*RI and 0.2 µg/µl PprA (left, WT; right, three amino acids mutated PprA) with heat shock treatment at 90°C for 0-60 min were applied to the wells of an agarose gel.

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The Effect of *Aspergillus puulaauensis* on Synthetic Consolidants Used in Restoration-Conservation Practice

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The influence of synthetic materials used in conservation-restoration of the cultural heritage artefacts on these objects has been already studied. However, compatibility, degradability and long-term effects of these materials are not fully understood [1,2,3,4,5]. They are chemically and technically different from traditional materials (with possible practical advantages), which raises question about ethical attitude towards cultural heritage that should stay materially inviolable.

Fungi are causing visible or/and structural damages on art objects, especially due to their ability to grow at low relative humidity (RH) and production of various enzymes and organic acids. Our study focused on the xerotolerant fungus *Aspergillus puulaauensis*, which was isolated from canvas paintings. The tested materials (Lascaux Acrylic Glue 498 - butyl-methacrylate-dispersion, Beva 371 - mixed material and Regalrez 1094 - hydrocarbon resin) were applied onto glass surfaces to test their susceptibility against the growth of the selected fungal strains. The materials were inoculated with fungi either immediately after application and drying, or after artificial ageing. The latter was achieved by exposure of the slides for 3 weeks (300 hours) to UV-B light at room temperature (22°C). Inoculated materials were incubated for 2 months in moist chambers with RH 90% and 22°C. The growth of the fungus was confirmed morphologically using stereomicroscope, and by DNA isolation and re-sequencing of selected genetical markers (β-tubulin). The chemical changes of studied materials were assessed with FT-IR Photoacoustic Spectroscopy (PAS), which showed some changes in the molecular structure of analysed materials, mainly visible as appearance of the band between 3100 and 3500 cm^{-1}, typical of O-H vibrations, appearance of band between 1700 and 1550 cm^{-1}, typical of either water or unsaturated chain vibrations and appearance or changes in carbonyl band vibrations. All these changes reveal hydrolytic and oxidative processes in synthetic materials caused by fungal degradation. The acrylic resin (Lascaux 498HV) was the least affected.

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Archaeal biofilms: Exopolysaccharide composition, size and synthesis in *Sulfolobus acidocaldarius*

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Biofilms are defined as microbial communities embedded in a hydrated, self-produced matrix of extracellular polymeric substances (EPS). Although Archaea are ubiquitous and believed to exist predominantly in the biofilm mode, knowledge about archaeal biofilm formation, structure, EPS composition and synthesis is limited [1]. In this study, we investigate biofilms of the thermoacidophilic (76°C, pH 3), aerobic Crenarchaeon *Sulfolobus acidocaldarius* [2] with a special focus on exopolysaccharide (PS) composition, size and synthesis. PS constitute a major EPS component beside proteins and extracellular DNA, suggesting an important role in *Sulfolobus* biofilms. In order to perform detailed structural PS analyses, high amounts of biomass were required. Therefore, archaeal biofilms were grown on membranes, floating on the surface of liquid medium. EPS components were isolated and quantified [3], and the monomeric composition of *S. acidocaldarius* MW001 PS was defined by acidic hydrolysis, chromatographic separation and mass spectrometry. Size exclusion chromatography was used to determine the nominal weight of PS species. A gene cluster comprising 14 glycosyltransferases and 7 membrane proteins likely involved in PS synthesis was identified in *S. acidocaldarius* [4]. Several deletion mutants have been constructed and phenotypes of wildtype and mutant strains were compared using the established analytical methods. The current insights in the composition and size of the *S. acidocaldarius* PS, the function of selected proteins encoded by the PS gene cluster as well as a model for PS synthesis and export will be presented.

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A novel extremophilic endo-glucanase from *Acidocaldarius* sp. for sugars recovery from municipal sludge, toward a bio refinery approach

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Municipal wastewaters sludge contain huge quantities of organic materials such as cellulose, mostly derived from the discharge of toilet paper in public sewers. The recovered bio-solid residues consist of a relevant cellulosic fraction for the valorisation of which only few studies in the literature are currently reported. Today the unstable global energy market highly pursues the exploration of low-cost feedstocks for the production of biofuels as important alternatives to fossil and petroleum-based products. The main goal of this study was to investigate the valorisation of the cellulosic fraction of the primary sludge through its enzymatic hydrolysis. The use of enzymes from extremophilic microorganisms, which are highly robust and resistant to numerous toxic pollutants present in the wastewater sludge (drugs, detergents, surfactants), overcomes the drawbacks caused by the use of conventional enzymes not resistant to the harsh conditions of the industrial processes. Our research has been focused on the utilization of a cellulase from an extremophilic bacteria classified as *Alicyclobacillus* sp. isolated from Lagoa das Furnas hot springs (São Miguel-Azores, Portugal). This cellulolytic microorganism exhibited high level of an extracellular endo-glucanase. The enzymatic crude extract showed its optimal activity at 65°C and pH 4.0 and a relevant stability around these values of temperature and pH. The application of the partially purified enzyme was studied for simple sugars recovery from primary sludge and primary cellulosic sludges from urban wastewater treatment plants. The cellulase extract was utilized in enzymatic cocktail with different quantities of commercial β-glucosidase. The glucose yields achieved were 5.30 and 8.39 g/L using the primary sludge and the primary cellulosic sludge respectively, results comparable to those achieved with the commercial enzymatic mixture.

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Microbial metabolic profile and viral auxiliary metabolism of the serpentinite-hosted prony bay hydrothermal field (New Caledonia)

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The hydration of ultramafic rocks exhumed from the upper mantle, a process known as serpentinization, produces highly reduced alkaline fluids enriched in dihydrogen (H_2). The latter can react with inorganic carbon to abiotically form methane, formate among short chain hydrocarbons and organic acids. Accordingly, serpentinizing environments can provide substantial energy and carbon sources for the inhabiting microbial communities[1]. The knowledge on microbial ecology in these ecosystems has significantly grown in the past 20 years, revealing potential adaptative processes and metabolisms linked to serpentinization byproducts[2] or involved in sulfur compounds oxidoreduction[3,4]. In contrast, little is known about viral diversity and their influence on metabolisms of their host in serpentinite-hosted ecosystems.

In this study, we examined the potential metabolisms of microbial communities inhabiting an alkaline serpentinite-hosted hydrothermal field at the Prony Bay (New Caledonia)[5]. The Prony Bay hydrothermal field (PBHF) hosts submarine carbonate chimneys emitting alkaline (pH>11), hot (42°C), and anoxic fluids enriched in H2, CH4, and abiotic organic compounds. Here, we presents the first results of five metagenomes from PBHF. Microbial diversity and their potential metabolic profiles were studied using gene-centric approach. Moreover, more than 310 microbial genomes were assembled from the metagenomes to identify key players of metabolic pathways. In addition, viral taxonomic diversity, hosts range and auxiliary metabolic genes were investigated in our metagenomes. These results will allow us to discuss importance of virus-microorganisms interaction on PBHF ecology and biogeochemistry.

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Engineering of *Sulfolobus acidocaldarius* for hemicellulosic biomass utilization

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Lignocellulosic biomass is a plant dry matter, is considered a second-generation biofuel producer. It includes various substances, including agricultural wastes, forest residues, and crops. To utilize lignocellulosic biomass as a biofuel, saccharification of cellulose and hemicellulose is essential. While cellulose is composed of glucose only, hemicellulose consists of various sugars including xylose, arabinose, glucose, and galactose. *Sulfolobus acidocaldarius* can be a good candidate for hemicellulose biofuel production as this archaeon simultaneously utilizes various sugars. However, *S. acidocaldarius* has to be manipulated because the enzyme that breaks down hemicellulose is not present in this archaeon. In this study, we engineered *S. acidocaldarius* to utilize xylan as a carbon source by introducing xylanase and β-xylosidase amplified from *Saccharolobus solfataricus*. Heterologous expression of β-xylosidase enhanced *S. acidocaldarius* degradability and utilization of xylooligosaccharides (XOS), but the mutant still failed to grow when xylan was provided as a carbon source. Also, *S. acidocaldarius* with single introduction of xylanase was shown to hydrolyze xylan but still cannot grow when xylan was provided as a carbon source. It is revealed that *S. acidocaldarius* can degrade xylan into XOS by introducing xylanase, but no further degradation proceeded following sole reaction. When xylanase and β-xylosidase were introduced into *S. acidocaldarius*, the constructed mutant was able to grow in the presence of xylan, suggesting that *S. acidocaldarius* successfully utilizes xylan in the synergy between xylanase and β-xylosidase.

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Diversity of microbial communities of methane pokmarks, gotland depression and the gulf of finland of the baltic sea

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It is now known that methane in deep-sea volcanoes and pokmarks around the world ocean have several sources of origin, thermogenic and microbial, the methane of microbial origin appears due to anaerobic decomposition of organic matter by prokaryotes closer to the bottom surface. The main producers of methane are methanogenic archaea, in addition, recent studies have revealed the production of methane by various different organisms phytoplankton, cyanobacteria [1,2]. Baltic sea is rich in these structures and their study gives a greater understanding of the functioning and diversity, especially in the eastern Baltic where such structures are less studied. During the 51th cruise of the R/V Akademik Sergey Vavilov. samples were taken from two elongated pokmarks, one point of the ASV 51059 Gotland Depression Sweden and the second ASV 51076 Gulf of Finland Russia. For genetic analysis, 10 samples were taken from different depths of each geological column. The saturation of bottom sediments with gases (methane, oxygen, carbon dioxide and nitrogen) was studied. As a result, the communities of methane pokmark of the Gotland Depression and the Gulf of Finland are very diverse and are represented by many taxa, and depending on the depth, the structure changes significantly. In general, the communities of the two Pokmark are represented by more than 25 phylum of archaea and bacteria, the dominant groups of bacteria are: the phylum Actinobacteriota, the dominant class of Actinobacteria from 2 to 24%, and other representatives of the phylum Actinobacteriota, which range from 0.1 to 5%. The phylum Caldatribacteriota class JS1 is the dominant phylum in most of the deep layers of the sampled samples and ranges from 0.1 to 34%. There are also representatives of the phylum Desulfobacterota class Desulfobacteria, they range from 0.7 to 7% in all samples. The community of archaea is represented by groups of Asgardarchaeota class Lokiarchaeia (1-1.5%) Crenarchaeota class Bathyarchaeia (0-1%) Phylum Thermoplasmatota class Thermoplasmata is about 1% Nanoarchaeota class Nanoarchaeia is 0.7 -1%. Also was found an unknown genus of marine Mycobacterium from the phylum Actinobacteria. According to the data it was shown relationship between the methane concentration and the JS1 phylum.

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

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There's an increasing demand for solutions to move away from non-renewable resources, and enzymes are an environmentally viable alternative to oil-based chemistry in industrial processes. To further advance this transition, in RadicalZ we will reduce the time required for enzyme discovery and development.

Enzymes that can decompose synthetic plastics such as polyethylene terephthalate (PET) are urgently needed. However a bottleneck remains due to a lack of techniques for efficient screening of good candidates. The project RadicalZ aims to reduce the time required for enzyme discovery and development using ultrahigh-throughput screening, where enzyme libraries compartmentalized in water-in-oil emulsion droplets are assayed.

Here we describe a pipeline for the ultrahigh-throughput screening of evolved thermophilic enzymes using fluorescence activated droplet sorting, in which we selected fluorescein dibenzoate (FDBz) as the fluorogenic probe. FDBz is not a highly reactive substrate of common esterases, hence it has a low fluorescence background in cell lysates. However, FDBz has PET-like ester bonds linked with a benzene group and can likely be hydrolysed by PETases to generate fluorescein monobenzoate. The pipeline comprises four steps: 1) generation of a library of PETase mutants by error-prone PCR. 2) Incubation of droplets encapsulating single cells and FDBz. 3) screening and sorting of droplets to obtain improved PET-degrading enzymes. 4) Sequencing of the evolved enzyme and further evolution cycles to improve activity.

Thermogutta terrifontis esterase 2 (TtEST2) [1], is being used as a test system to establish the microfluidic experiments. TtEST2 differs from most enzymes of the α/β-hydrolase family 3 as it lacks most of the 'cap' domain and its active site cavity is exposed to the solvent allowing bulkier substrate - like PET - to be accepted. TtEST2 is active against FDBz which is a good indication of its PET digesting capability [2] and is currently being evolved to improve its activity to bulkier substrates.

Furthermore, five potential PETase sequences have been identified in the HotZyme Exeter DNA database (contains novel thermophilic genomes and thermophilic metagenomes) and in other public databases which also have shown activity against FDBz.

This preliminary work serves as a foundation for the evaluation of PETase activity, using PET film and fibres, with the goal to develop a label free microfluidics screening method for directed evolution of these industrially useful enzymes.

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Transcriptional regulation of aromatic amino acid biosynthesis operons in the hyperthermophilic archaeon *thermococcus kodakarensis*

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Thermococcus kodakarensis KOD1 is a hyperthermophilic archaeon isolated from a solfatara on Kodakara Island, Kagoshima, Japan. *T. kodakarensis* is strictly anaerobic and heterotrophic, and can utilize amino acids, peptides, pyruvate and a number of polysaccharides such as starch. The genome of *T. kodakarensis* is a single circular chromosome consisting of 2,088,737 bp and 2,306 predicted ORFs were identified. Genetic systems have been developed in this organism, allowing genetic analyses on metabolic enzymes and regulating factors. We have been interested in the mechanisms of transcriptional regulation of genes related to amino acid biosynthesis. We have identified a transcriptional regulator, Tar (Thermococcales aromatic amino acid regulator), that activates the transcription of the tryptophan biosynthesis gene operon (*trp* operon) in *T. kodakarensis*[1]. Tar recognizes the sequence TGGACA-N_8-TGTCCA, and this motif can also be found in the promoter regions of the chorismate biosynthesis operon (*aro* operon) and the phenylalanine/tyrosine biosynthesis operon (*phe*/*tyr* operon). *In vitro* and *in vivo* data indicate that Tar also activates both of these operons. The *trp*, *aro* and *phe*/*tyr* operons are clustered together on the *T. kodakarensis* genome and interestingly, an operon encoding all components of the histidine biosynthesis pathway (*his* operon) is located adjacent to the *trp* operon. The Tar binding motif was also found in the upstream region of the *his* operon. We also found that Tar binds to a DNA fragment that contains this motif within the *his* operon upstream region. The results suggest that the *his* operon at least partially shares a common mechanism for transcriptional regulation with the *trp*, *aro* and *phe*/*tyr* operons in *T. kodakarensis*. We are now examining mechanisms that would add specificity to the regulation of each operon.

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Marinomonas sp. Ef1: a platform to study cold-active enzymes and their biotechnological exploitation

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Cold-active enzymes are produced by psychrophilic organisms to survive in cold environments. The high activity at low temperatures and the thermolability of these enzymes make them attractive both for the study of cold adaptation mechanisms and for their potential biotechnological applications [1, 2]. Polar marine microorganisms represent a gold mine for the discovery of new cold-active enzymes endowed with unusual biochemical and structural features.
Marinomonas sp. ef1 is an Antarctic marine bacterium able to grow in the temperature range from 4 to 22°C [3]. Genomic analysis of this bacterium reveals sequences coding for a wide array of hydrolytic enzymes exploitable for biotechnological applications and biochemical studies. Here we report the structural and functional characterization of a panel of hydrolytic enzymes identified in the genome of *Marinomonas* sp. ef1. These enzymes belong to esterase and glycoside hydrolase 1, 3 and 42 [4] families, are active at low temperatures, but present different thermal stability suggesting different mechanisms of cold adaptation. The atypical combinations of cold activity, substrate specificity and thermal stability of these enzymes pave the way for their use in the valorization of raw materials such as cheese whey and marine polysaccharides.

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Kavart acid tailing as a source for new metal resistant bacteria with a heavy metal bioremediation potential

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Development of the mining industry and formation of the tailing dumps increase the release of heavy metals to the environment. Metallophilic microbes, able to thrive in high concentrations of toxic metals could have important applications in bioremediation of metal-contaminated environments. The goal of the current work was isolation and identification of acidophilic heavy metal resistant bacteria from sludge samples of the Kavart mine tailing in the Syunik region of Armenia, and investigation of their potential for bioremediation of the contaminated environment.

The samples from the Kavart mine tailing are characterized by acidic pH (around 2.5) and high concentrations of heavy metals. For the enrichment of metal-resistant bacteria different media with pH varying from 3.0 to 5.5 were used. As the result, 10 Gram-negative and Gram-positive, acidophilic and metal-tolerant bacteria were isolated, and based on 16S rRNA gene sequence analyses they were identified as members of the *Acidiphilium*, *Acidocella*, *Pseudomonas*, *Rhodococcus* and *Sinamonas* genera. The most metal resistant strains, *Rhodococcus* sp. KT1-2 and *Acidocella* sp. K2-4, both with MIC values for Cu(II), Zn(II) and Ni(II) of 21, 29.5 and 46 mM, respectively, were selected for more detailed studies. The genomes of the strains were sequenced using Illumina paired-end technology, and the data assembled and analyzed using PATRIC 3.6.12 and RAST version 2 web tools. The draft genomes of strains KT1-2 and K2-4 constituted 9.7 and 3.1 Mb, respectively. The results from average nucleotide identity (ANI) analysis and Genome-to-Genome Distance Calculator revealed a two-way ANI value of 99.1% and 91.7% and a dDDH value of 91.7% and 25.1% respectively, between *Rhodococcus* sp. KT1-2 and its closest relative, *Rhodococcus hoagie* strain DSM20295, and between *Acidocella* sp. K2-4 and its closest relative, *Acidocella aromatica* strain DSM 27026. The latter result indicates that K2-4 represents a novel species. Both strains contain metal resistance genes, which highlights their potential as new tools for bioremediation.

Acknoledgements. The work was supported by MSECS RA Science Committee grant 19YR-1F065 and 21SCG-1F016.

Unveiling the role of Dps and EndoIII for DNA protection and repair in Deinococcus radiodurans upon exposure to genotoxic stress

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Deinococcus radiodurans (*Dr*) is known for showing great resistance to ionizing radiation, desiccation and to oxidative stress amongst other extreme conditions[1]. This bacterium possesses different groups of proteins which are involved in the response to several stress agents by either protecting the DNA from suffering damage or repairing efficiently the damaged DNA. In this work we focused on two Dps (DNA binding protein under starved conditions) present in *Dr* (Dps1 and Dps2), which are able to bind and protect DNA against damage[2, 3] and on three Endonuclease III proteins (EndoIII-1, EndoIII-2 and EndoIII-3) which are responsible for repairing the damaged DNA through the Base Excision Repair (BER) pathway[4]. To understand the role of these proteins and their possible interplay, we constructed and studied the resistance to stress of both single and double knockout mutants, of each of these proteins, through the Tripartite Ligation Method, using Overlap PCR. The induced stress was the exposure to UVC radiation, hydrogen peroxide and methyl viologen, and the stress response was compared with the *wild type* bacterium. Moreover, the interaction between Dps1 and DNA was studied at molecular and structural levels and we have determined the structure of this complex using Cryo-Electron Microscopy – Single Particle Analysis.

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Funding acknowledgements

- INSTRUCT-ERIC-Access Proposal, PID: 12696- „'Unveiling the molecular details of the Dps-DNA complex interaction, using Single particle analysis' - Electron Microscopy Unit, University of Helsinki, Finland
- Fundação para a Ciência e Tecnologia, Project- PTDC/BIA-BQM/31317/2017, Metals in the radiation resistance bacterium *Deinococcus radiodurans*, its role in protection up to future applications.
- European Union's Horizon 2020 research and innovation programme under grant agreement No 857203- IMpaCT - Imaging life from Molecules to cells - building knowledge on Cryo-electron microscopy methodologies at ITQB NOVA – www.itqb.unl.pt/impact.

Cultivation of "Microbial Dark Matter" From an Acid Mine Drainage Environment

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"Microbial dark matter" describes prokaryotic organisms prevalent in nature but are unable to be cultivated in laboratory settings. It is estimated that only 0.1-1 % of all microorganisms and 50% of archaeal phyla do not have a single cultivated representative [1]. Recently, several taxa of prokaryotes were identified, which fit into the "microbial dark matter" category, from the highly acidic and metal contaminated sites of the abandoned copper mine Parys Mountain, Wales, UK [2,3]. 8 different phylotypes of *Ca*. *Micrarchaeota* (*Ca*. Microcaldota) were identified across 2 sites, including *Ca.* Mancarchaeum acidiphilum (Mia14-like) and ARMAN-2-like archaea [2,3,4]. The focus of this work was the cultivation of these "microbial dark matter" groups to get further insights into their physiological traits, to recognise the range of possible hosts and host:symbiont interactions and to understand their role in acidic environments. Enrichment cultures composed of several different liquid media types and containing a wide variety of substrates and supplements were used. Compositional changes within the enrichment cultures were monitored using 16S rRNA metabarcoding and next generation sequencing. Additionally, uncultured prokaryotic groups inhabiting AMD were accumulating in enrichment cultures established with Parys Mt samples. Enrichment cultures found to contain archaea of the phylum *Micrarchaeota* and bacterial taxa RCP1-48 and Sva0485 and others, which although prevalent across acid mine drainage environments, are to date, unculturable and possess unclear physiological traits and roles in the environment. This study expands our knowledge on biology of "microbial dark matter" taxa in hyper acidic environments.

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An archaeal phosphoglycolate salvage pathway

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Many autotrophic organisms that utilize the Calvin-Benson-Bassham (CBB) cycle harbor metabolic pathways to salvage 2-phosphoglycolate (2-PG), a wasteful product generated by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The glycolate pathway, or the C2 pathway, of photorespiration and the glycerate pathway have been reported as 2-PG salvage pathways[1,2]. It has been presumed that the occurrence of 2-PG salvage is linked to the CBB cycle and oxygenic photosynthesis. Here we demonstrate that the enzyme activities required for converting 2-PG to glycine or serine, 2-phosphoglycolate phosphatase, glycolate dehydrogenase, glyoxylate:amino acid aminotransferase, glycine cleavage system together with a previously identified serine hydroxymethyltransferase[3], are present in the hyperthermophilic archaeon *Thermococcus kodakarensis*. The genes responsible for the activities were identified by biochemical and/or genetic analyses. As the Rubisco in *T. kodakarensis* functions in the pentose bisphosphate pathway and not in the CBB cycle[4], 2-PG salvage in this organism emerged independent of the CBB cycle. Previous studies suggested the presence of a metabolic link from serine to 3-phosphoglycerate in *T. kodakarensis*[3]. Together with the pathway identified here that converts 2-PG to serine, this suggests that *T. kodakarensis* can convert 2-PG to 3-phosphoglycerate, a conversion that resembles the glycolate pathway of photorespiration.

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Nitrifier diversity in geothermal springs – who likes it hot?

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Microbial communities in geothermal waters are composed of various, phylogenetically diverse microorganisms adapted to life under extreme conditions such as temperature and pH. Many previous studies have established that the abundance and diversity of microorganisms are usually reduced when compared to other aquatic habitats [1]. The primary objective was to identify, enrich and characterize nitrifying microorganisms (ammonia and nitrite oxidizing, and comammox microorganisms) from Croatian geothermal springs and wells. We used *amoA* gene quantitative PCR to quantify ammonia oxidizer communities in geothermal samples, and thereafter select localities for nitrifier enrichment experiments. One spring (Antunovo vrelo) and two wells (TS Stubicke and Krecaves) have been selected for sample collection, and enrichment experiments. AOA of the genera *Candidatus* Nitrosotenuis and *Candidatus* Nitrososphaera as well as comammox *Nitrospira* were detected to occur at high abundance in the selected samples, based on 16S rRNA gene amplicon sequencing and *amoA* gene qPCR data. However, enrichment procedures showed only limited success in respect to AOA enrichment, while comammox *Nitrospira* did seem to prevail and grow in a fraction of enrichment cultures. Interestingly, ammonia oxidation also occurred in enrichments in which *amoA* gene qPCR consistently yielded negative results, indicating a enrichment of novel, hitherto unknown ammonia oxidizers. Further exploration of the enrichment cultures by metagenome sequencing is underway.

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Sodium Trehalose symporter contributes to anhydrobiosis in Pv11 cells

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Water is an essential substance for life. Lack of intracellular water causes protein denaturation and cell membrane aggregation, leading to death in the worst cases. However, larvae called *Polypedilum vanderplanki* and cultured cell lines (Pv11) exhibit extreme desiccation tolerance [1]. Trehalose is known to maintain cell function by stabilizing cell membranes and protecting the structure of biological components [2]. Pv11 transports trehalose into and out of the cell using a trehalose transporter. Recently, STRT1 (Sodium-coupled Trehalose Transporter1) was identified as a Na^+/trehalose transporter. Using the CRISPR/Cas9 system already established for Pv11, we established a cell line in which the *Strt1* gene was knocked out (*Strt1*$^{-/-}$) [3].

The desiccation protocol for Pv11 is divided into three steps: “trehalose treatment”, “desiccation”, and “rehydration”. The survival rate of the *Strt1*$^{-/-}$ cell line after rehydration was significantly lower than that of WT cells. Comparison of trehalose uptake into cells during trehalose treatment of WT and *Strt1*$^{-/-}$ using HPLC showed no significant change in both cell groups. However, *Strt1*$^{-/-}$ showed delayed intracellular Na^+ efflux after rehydration compared to WT using SBFI-AM, a fluorescent indicator for Na^+. Furthermore, compared to WT, *Strt1*$^{-/-}$ was rounded after rehydration and could not maintain the normal cell shape. Intracellular trehalose accumulates in Pv11 after trehalose treatment, and intracellular Na^+ concentration increases after desiccation. These results suggest that STRT1 is a trehalose transporter and maintains cell homeostasis by rapidly removing Na^+ after rehydration.

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Sodium-dependent trehalose symporter contributes to anhydrobiosis in Pv11 cells

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Water is an essential substance for life. Lack of intracellular water causes protein denaturation and cell membrane aggregation, leading to death in the worst cases. However, larvae *Polypedilum vanderplanki* and cultured cell lines (Pv11) exhibit extreme desiccation tolerance [1]. Trehalose is known to maintain cell function by stabilizing cell membranes and protecting the structure of biological components [2]. Pv11 transports trehalose into and out of the cell using a trehalose transporter. Recently, *Strt1* (Sodium-coupled Trehalose Transporter1) was identified as a Na^+/trehalose transporter. However, the biological function of this transporter has not been demonstrated, and its role in desiccation tolerance is unknown. Using the CRISPR/Cas9 system already established for Pv11, we established the *Strt1* knocked-out cell line (*Strt1*$^{-/-}$) [3]. Pv11 WT(Wild Type) cell line and *Strt1*$^{-/-}$ do not change their survival rate during normal culture and before desiccation. However, the survival rate of the *Strt1*$^{-/-}$ after rehydration was significantly lower than that of WT. Comparison of trehalose uptake into cells during trehalose treatment of WT and *Strt1*$^{-/-}$ showed no significant change. However, *Strt1*$^{-/-}$ showed delayed intracellular Na^+ efflux after rehydration compared to WT using a fluorescent indicator for Na^+. Furthermore, *Strt1*$^{-/-}$ was rounded after rehydration and couldn't maintain the normal cell shape. In Pv11, Intracellular trehalose accumulated and intracellular Na^+ concentration increased after desiccation. These results suggest that STRT1 is a transporter and maintains cell homeostasis by rapidly removing Na^+ after rehydration.

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Discovery and functional characterization of a novel thermostable and promiscuous ketoreductase from a hot spring metagenome

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The (meta)genome mining-based search of novel thermostable hydroxysteroid dehydrogenases (HSDHs), enzymes able to regio- and stereoselectively oxidize/reduce steroidal compounds,[1] has recently led us to the discovery of a novel Short-chain Dehydrogenase/Reductase (SDR), named Is2-SDR. This enzyme, found in an Icelandic hot spring metagenome, shared a high sequence similarity with HSDHs, but, with our disappointment, showed no activity in the oxidation of steroid substrates, e.g., cholic acid.

Despite that, Is2-SDR proved to be a very active and versatile ketoreductase, being able to regio- and stereoselectively reduce a diversified panel of carbonylic substrates, including bulky ketones, α- and β-ketoesters, and α-diketones of pharmaceutical relevance.[2-4]

Moreover, it showed a remarkable thermostability, with an apparent melting temperature (T_M) around 75°C, as determined by circular dichroism analysis, and no significant decrease of the catalytic activity even after 5 h at 80°C. Is2-SDR showed also a broad tolerance to both water-miscible and water-immiscible organic solvents, and was successfully immobilized on the Eupergit C® resin, to lead its synthetic application in the future from batch to flow bioprocesses.

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Extremophile endolithic communities in the pacific peruvian desert

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In the tropical region of South America, Peruvian coastal desert is located at the central Pacific western.The hypersaline wetland ecosystems studied were the Puerto Viejo at 69 km south of Lima, the Salines of Huacho 110 km north of Lima and Salines of Chimbote, Ancash. Standard cyanobacterial and algal collections were carried out between 2010 and 2020 with physical chemical parameters as well as the morphospecies identification with microphotography sequences of vegetative and reproductive stages of cyanobacteria and algae. The wetlands are exposed to annual fluctuating hydrological regime of flooding and desiccation periods with saline crust formation. The water budget is governed by groundwater discharges and the evaporation leads to dry out by late summer and fall seasons with precipitation of evaporitic minerals such as gypsum, halite, aragonite, etc. Colonization and developmental stages of the colonial cyanobacteria *Pleurocapsa entophysaloides* embedded in polysaccharide sheath to avoid desiccation is reported. The colonies became embedded in a porous crystalline matrix as crypto and chasmolithic species colonizing soil cavities and fissures with a notorious blue green layer (0.5- 2.5 cm beneath the crusty soil surface). Besides, the growth embedded in a saline soil and reproductive strategies with the baeocyte formation (multiple fission) as well as its release favored the spreading in the extreme lithified saline soil. In addition, the upper surface layer where solar radiation is intense brown pigmentation(scytonemin) was recognized. Between other associated phototrophs of the endolithic communities were *Rhizoclonium hieroglyphicum, Calothrix crustacea* ,and *Tetraselmis contracta* (resistant spores). Then, *P. entophysalis* is a dominant extremophile indicator of the saline soil with successful colonization due to its survival strategies under the climate change predictions leading to an increase in intensity and frequency of droughts across the desert tropical landscape.

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Development of rna capping enzymes for mrna vaccine

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Cap1 is a signature of self mRNA and any other structure less than cap1 could course innate immune response in the host [1, 2]. Cap1 structure of the mRNA has utmost importance in the preparation of mRNA vaccine. RNA triphosphatase, RNA guanylyl transferase, guanine N-7 methyl transferase, and 2-O methyl transferase are the four different enzymatic activities required for the preparation of cap1 structured mRNA. Vaccinia virus (VACV) mRNA capping enzymes are widely investigated for many decades since they produce relatively large quantity of cap1 structured mRNA [3, 4, 5]. Vaccinia D1 (844-aa)-D12 (287-aa) heterodimer consist of the core enzymatic activities required for the cap0 structure and additional VP39 (333-aa) contains the 2-O methyl transferase activity which is essential for the preparation of cap1 structured mRNA. VP39 can be expressed in *E. coli* at 100 mg/L and purified in large quantity without many complications. However, maximum yield for D1-D12 purification that has been achieved so far is 2.4 mg/L [5]. We clone D1-D12 from two different vaccinia strains namely Ankara and Western Reserve into the pET vector expression system (Novagen). We found that D1-D12 soluble expression from Western Reserve strain is three-fold more than Ankara stain. In addition, we were able to achieve 5 mg/L D1-D12 purification employing pET vector expression system which is about two-fold higher than published data [5].

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A Novel Alkane degradation enzyme of *Geobacillus kaustophillus* HTA426 isolated from the Mariana Trench

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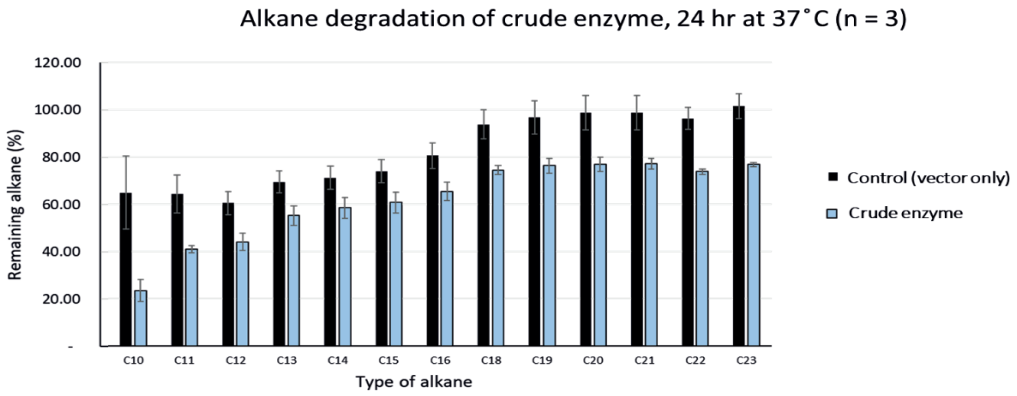
A thermophilic bacterium, *Geobacillus kaustophilus* HTA426, isolated from the Mariana Trench (depth 10,897m, 55°C), was found by chance capable of degrading alkanes at 60°C. After 10 days of cultivation at 60°C HTA426 degraded alkanes of 10 to 16 carbons to around 50% or lower. Surprisingly, it revealed that none of the genes encoding currently known alkane hydroxylase and monooxygenase is existed in its genome. In this study, we investigate alkane degradation ability of HTA426 and potential genes responsible for degradation. One of the candidate genes encoding, a putative small subunit of nucleotide ribonuclease, was cloned and expressed in *E. coli* BL21 (DE3). Crude extract of the recombinant protein showed reduction of 10 to 16 carbon alkanes, especially with 10 to 12 carbons by 24 hr at 37°C. This finding may lead to uncover a new evolution pathway of alkane hydroxylase gene under chaotic condition of high temperature and high pressure.



Comparison of remaining alkane between control and crude enzyme

NanoDSF-driven stabilisation of a novel psychrophilic enzyme

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Psychrophilic enzymes present significant promise in reducing the required temperatures for various industrial processes which are currently performed at high temperatures. They are naturally active at lower tempertures and their use can achieve significant energy savings which is a hot-topic in the current global climate. However, implementing psychrophilic enzymes in industrial settings comes with a host of unique challenges, one of which is their tendency to be unstable at temperatures normally present during preparation and storage of enzymes. In this study a novel enzyme, Enz2, was identified from a metagenomic DNA database from cold environments, using the bioinformatic tools pBLAST and Clustal Omega. The Enz2 was recombinantly expressed in *Escherichia coli* and purified using the chromatographic methods of nickel affinity chromatography and gel filtration. Preliminary biochemical studies have revealed some desirable kinetic properties of industrial interest. However the Enz2 was also found to be unstable at ambient temperatures and have low solubility
The strategies employed to overcome the low protein stability and solubility will be discussed. NanoDSF is a high-throughput dye-free technique which monitors intrinsic tryptophan fluorescence over a temperature gradient allowing for rapid elucidation of protein melting temperatures in a range of different buffer environments. However, Enz2 was not initially suitable for nanoDSF studies due it's maximum solubility being below the minimum concentration requirement of ~1 mg/mL. To overcome this problem a a method has been developed to stabilise Enz2 by screening various buffer and additive conditions for the long term retention of its enzymatic activity. This approach has improved the solubility of Enz2 to allow the use of nanoDSF to further optimise its properties. This has resulted in a >10X improvement in purification yields and 30X increase in maximum solubility. The successful stabilisation of Enz2 has allowed further biochemical studies to be carried out and evaluation of its potential industrial applications.

Detection of enzymes with lipolytic activity in thermophilic microorganisms isolated from ash and porous rock of the Mexican volcano PopocatÉpetl

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The Mexican volcano Popocatépetl is located between the limits of the states of Puebla, Morelos and Estado de México. It is part of the Trans-Mexican Volcanic Belt, which is the volcanic arc that extends over the southwestern margin of the North American Plate as a result of the subduction of the Rivera and Cocos plates along the Acapulco Trench[1]. Popocatepetl is an active volcano permanently monitored by the National Centre for Disaster Prevention. The aim of this work was to isolate and identify thermophilic bacteria in ash and porous rock samples from the foothills of Popocatepetl volcano and to evaluate their lipolytic capacity. Molecular identification of five strains isolated at 55 and 65 °C on LB medium was performed. According to the sequence alignment of the 16S rRNA gene, strains were identified as *Bacillus thermocopriae* strain C255, *Caldibacillus* sp. strain C365, *Parageobacillus caldoxylosilyticus* strain C165, *Aeribacillus pallidus* strain P455 and *Anoxybacillus caldiproteolyticus* strain P265. The pH of the ash and porous rock was 6.5 and 6.3, respectively. The strain that showed the highest lipolytic activity was *Bacillus thermocopriae* C255 at 50 °C with *p*-nitro phenol laurate as substrate. In the *in-situ* activity analysis, a 66 kDa band with lipolytic activity in the presence of MUF-butyrate was found in the intracellular fraction.

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Biochemical characterization of a novel acidic laminarinase derived from jermuk hot spring metagenome

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In the light of current environmental challenges, such as the depletion of fossil fuel reservoirs and the resulting anthropogenic climate change, a global transition to a more sustainable biobased products and processes is highly demanded. Recently, increasing attention is being drawn to macroalgae as an ideal biomass source with high levels of valuable carbohydrates and low to zero lignin content [1]. In macroalgae, the laminarin content ranges from 1-25 % of the total weight [2]. Laminarinases, which catalyse the hydrolysis of β-1,3-glycosidic linkages of laminarin, are promising biocatalysts for the bioconversion of this substrate to industrially relevant compounds.

The ORF Jermuk-lamM was identified by a sequence-based screening of a metagenome obtained from a hot spring of Jermuk, Armenia. The product of the gene is a novel laminarinase (EC 3.2.1.39) sharing only 69 % amino acid sequence similarity to the GH16 family members available in the NCBI database. As of today, this is the only characterized endo-1,3-β-D-glucanase identified in the *Marinimicrobia* phylum. Jermuk-LamM is an acidic and thermostable enzyme. This recombinant enzyme was found to efficiently hydrolyse soluble and insoluble (1→3)-β-D-glucans as well as mixed-link (1→3,1→4)-β-D-glucans showing the highest activity toward laminarin.

Based on its properties this enzyme is a suitable candidate for a wide range of industrial applications including the production of biofuels, pharmaceuticals and various oligosaccharides [3].

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Karvachar geothermal spring as a sourse of Anoxybacillus karvacharensis sp. nov.

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The majority of *Anoxybacillus* species have been isolated from hot springs worldwide. The type species of the genus *Anoxybacillus*, *Anoxybacillus pushchinoensis* DSM 12423T, was first described by Pikuta *et al*. in 2000 [1]. Many *Anoxybacillus* species have been isolated from geothermal springs located in different regions of the Alpine–Himalayan orogenic belt. These observations inspired us to explore the microbial diversity in Karvachar (Nagorno Karabakh) geothermal spring, which is part of the Alpine–Himalayan orogenic belt. Twelve anoxybacilli strains have been isolated from water and sediment samples from Karvachar geothermal spring and identified based on 16S rRNA sequence analysis. They shared from 98.0 and 99.9% of 16S rRNA sequence similarity with those available in the GenBank database. Because of its high amylase activity [2], strain K1 was selected for further characterization based on phenotypic, chemotaxonomic and phylogenetic characteristics. The cells are straight, motile rods that are 0.2–0.4×2.3–7.2 μm in size. The strain is a Gram-stain-positive, moderately thermophilic facultative anaerobe with growth temperature range of 45–70°C (T_{opt} 60–65°C), growth range of pH 6–11 (optimum, pH 8–9). The strain was able to hydrolyse starch, casein and gelatin, was positive for oxidase and catalase, and reduced nitrate to nitrite, but was negative for H_2S production. The major cellular fatty acids were C15:0 iso, C16:0 and C17:0 iso (52.5, 13.6 and 19.6 % of total fatty acids, respectively). Strain K1 shares >99% 16S rRNA sequence similarity and a genomic average nucleotide identity value of 94.5% with its closest relative, *Anoxybacillus flavithermus* DSM 2641T, suggesting that it represents a separate and novel species, for which the name *Anoxybacillus karvacharensis* sp. nov. is proposed [3]. The type strain of *Anoxybacillus karvacharensis* is $K1^T$ (=DSM 106524T=KCTC 15807T).

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Thermophilic bacilli as industrial platforms: from one-carbon feedstocks to lactic acid production

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The use of fossil resources for energy and chemical production for decades has resulted in the current global warming, which threatens the stability of natural and human communities. Liquid-soluble one-carbon (C1) renewable compounds, such as methanol and formic acid, are a potential alternative to fossil fuels. These can be produced from renewable sources, such as atmospheric CO_2 or syngas [1]. To further convert C1 compounds into chemical commodities, microbial cell factories are preferred over traditional chemical and physical methods.

Therefore, using microorganisms to produce chemicals and fuels has become more popular over the years. Moreover, the development of genetic and metabolic engineering techniques facilitated the widening of product range and host range [2].

Lactic acid has multiple uses in the food, chemical, textile, cosmetics, pharmaceutical and polymers industries [4]. Among different potential production hosts, thermophilic organisms stand out for industrial applications. Firstly, their high optimal growth temperature reduces the energy needed for cooling the bioreactors, resulting in a reduction of the operation costs. Secondly, at thermophilic temperatures catalytic rates are higher than at mesophilic temperatures, which can result in faster growth and a more productive industrial process. Finally, the use of thermophilic microorganisms reduces the chance of contamination by mesophilic microorganisms [3]. In this poster, we will report on our progress on the use of thermophilic bacilli as cell factories to produce lactate using methanol as a carbon source.

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Expanding the synthetic biology toolbox of *Sulfolobus acidocaldarius*, a thermoacidophilic Crenarchaeon

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Industrial biotechnology has become increasingly interested in exploiting the vast potential present in the microbial world, as, e.g., natural producers and extremophiles can bear a significant advantage over conventional production hosts [1-4]. To unlock this potential, tools are needed to genetically engineer the host of choice. Typically, such a toolbox is not yet available for these organisms, like, e.g., for the thermoacidophilic Crenarchaeon *Sulfolobus acidocaldarius*, that grows optimally at pH 2-3 and 75-80 ºC. In this study, a semi-synthetic promoter library was created by randomizing specific parts of a native *S. acidocaldarius* promoter. The promoters were tested for *lacS* [5] expression, revealing promoters covering a 3-fold range of expression levels, with promoters outperforming the established *malE* promoter [6]. For efficient use of this promoter library in biosynthetic pathway construction, a Golden Gate based assembly method was developed allowing quick and efficient combination of promoters and genes in a pRN1-based *E. coli-Sulfolobus* shuttle vector [7].

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A new generation of biotechnological PHA production using thermophilic bacteria

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Polyhydroxyalkanoates (PHA) are microbial polyesters which are good alternative to petrochemical plastics. PHA are produced by many various bacteria, and they are completely biodegradable and biocompatible. However, these polymers are more expensive than petrochemical plastic.
A good solution that can reduce the price of biotechnological production, the use of waste carbon sources or the use of extremophilic producer. Ideally a combination of the two.
Extremophilic microorganisms are group of organisms which live and thrive in extreme conditions. This condition is key for reducing the price because the biotechnological process is possible in semi- or non-sterile condition.
PHA producers are often found among extremophiles. PHA production is most described among thermophilic and halophilic microorganisms.
We focused on new thermophilic producers of PHA using isolation. We designed an original isolation protocol leading to the isolation of PHA-accumulating bacteria from compost. The isolation protocol uses osmotic stress to selection PHA producing bacteria because PHA granules protected bacteria during osmotic stress. Compost and activated sludge are used as source of thermophilic producers. We have recruited over 40 promising producers of PHA. The producers were taxonomically classified based on 16S RNA sequencing. In the second part of the work, we tested their PHA production and selected the most promising ones. The production of PHA in selected thermophilic isolates reaches almost 60 % of cell dry biomass. The patented isolate culture of *Aneurinibacillus* sp. H1 is even capable of very interesting copolymers, for example with a high proportion of 4-hydroxybutyrate (up to 90 mol. %).

An *in silico* analysis of the alpha-amylase family gh119 and its relatedness to extremophilic family gh57

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In the sequence-based classification of glycoside hydrolases (GHs) and other carbohydrate-active enzymes, the CAZy database[1], the family GH119 represents one of the four α-amylase GH families[2]. The three remaining ones are families GH13, GH57 and GH126[3]. The family GH119 was established based on a study describing the α-amylase from *Bacillus circulans* (currently re-classified as *Niallia circulans*), a product of the *igtZ* gene, with a sequence exhibiting no obvious similarities to previously known α-amylases[4]. This family – with less than 40 members – belongs, in fact, to the smallest GH families[1]. In addition to a partial biochemical characterization of the representative IgtZ α-amylase from *Niallia circulans*[4] nothing, in fact, has been found in GH119 until now. There was only a single in silico study[5] predicting the catalytic domain structure, catalytic machinery and conserved sequence regions (CSRs) as shared with those determined in the second α-amylase family GH57, well-known by a high representation of extremophilic Bacteria and Archaea as sources of the enzymes[6,7]. The present bioinformatics study was undertaken in an effort to deliver a detailed analysis of all family GH119 members with regard to their domain arrangement, exact location of all five CSRs and unique sequence features. In order to perform a relevant comparison, the studied set of GH119 members was completed by all characterized family GH57 members. Based on the results of the presented work, one should be able to assign a hypothetical protein the either family GH119 or GH57 affiliation in cases when doubts might have arisen due to its intermediary sequence.

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Psychrophilic bacterial lipase for improved biocatalytic acylation process in oil industry

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Utilization of cold-active catalysts constitute one of the enhanced solutions in biotechnologies based on extremozymes. The current study addressed the development of a lipase-mediated catalytic system involved in silybin acylation using a cold-active lipase from the psychrophilic *Psychrobacter* SC65A.3 isolated from Scarisoara Ice Cave (Romania)[1]. The recombinant enzyme Lip2 was obtained by gene cloning and purified by affinity chromatography. Structural and functional characterization of the free and immobilized enzyme evidenced an improved stability and catalytic activity at 25°C, highlighting sequence elements related to low temperature adaptation. The transesterification process catalyzed by Lip2 led to an optimum silybin conversion by methyl decanoate and methyl palmitat acylating agents when using nano-support immobilized lipase biocatalyst. Alternatively, enzyme entrapped inside Na-alginate and K-carrageenan polysaccharide beads preserved full enzyme activity during 3 reaction cycles of silybin conversion by Lip2. This newly developed biocatalytic method for obtaining silymarin-enriched milk thistle oil by using cold-active lipase also constitute a valorization solution of waste biomass in oil industry.

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Cellular response of *sulfolobus acidocaldarius* to terbinafine, a proposed inhibitor of tetraether lipid biosynthesis

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Archaeal membranes are highly dynamic as their composition is dependent on environmental influences like nutrient availability, pH and temperature. The membrane lipids of archaea are ether-linked molecules, specifically dialkyl glycerol diethers (DGDs) and glycerol dialkyl glycerol tetraethers (GDGTs). Radiolabeling assays with the crenarcheaote *Thermoplasma acidophilum* have indicated the accumulation of DGDs upon treatment with terbinafine, a squalene epoxidase inhibitor in fungi[1, 2] , and it was suggested that this compound inhibits the tetraether synthase *(Tes)*. However, the early cellular response of archaea to terbinafine has not been studied yet. Here, RNA sequencing and lipidomics were used to elucidate the effect of terbinafine in *Sulfolobus acidocaldarius*. Accumulation of DGDs and depletion of GDGTs was observed when cells were treated with terbinafine. Additionally, a major shift in the saturation of caldariellaquinones was observed. Transcriptomic data indicates that terbinafine primarily targets the respiratory complex along with genes involved in motility, fatty acid biosynthesis and GDGT cyclization. Expression of the recently identified *Tes* was unaffected [3]. Combined, these findings suggest that respiratory stress is the preliminary response of *S. acidocaldarius* to terbinafine while targeting multiple genes involved in isoprenoid biosynthesis and saturation.

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Flow cytometry-based viability staining for bioprocess monitoring of *Sulfolobus acidocaldarius*

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The viability of *Sulfolobus acidocaldarius*, an extremophilic Archaeon thriving at 75 °C and pH 3.0, to date is still being determined by tedious and material-intensive plating assays that can only provide time-lagged results [1,2]. Although *S. acidocaldarius* and related species harbor great potential for the exploitation as production hosts and biocatalysts in biotechnological applications [3–6], no industrial processes have been established yet. During development and scaling of industrial bioprocesses, it is crucial to monitor the impact of process parameters on the cultivated organism – a task that cannot be fulfilled by traditional plating assays. Flow cytometry (FCM) combines the ability to analyze single-cell properties in a cell population with viability assessment via the use of fluorescent-active dyes [7–11]. In this study, commercially available fluorescent-active dyes applicable in *S. acidocaldarius* were identified. The dyes, fluorescein diacetate and concanvalin A conjugated with rhodamine, were suitable for viability determination via FCM. For showing the applicability of the developed tool for bioprocess monitoring a chemostat cultivation at 75°C and pH 3.0 was conducted. Over 800 hours, this novel FCM method successfully allowed monitoring of viability during applied pH drops. In my talk, I will introduce our exciting FCM method, paving the way for an untapped Archaeon into industrial application.

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Snow and glacial algae: ecology, biosignatures and prerequisites for living on icy worlds

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Amongst psychrophiles, snow and glacial algae are considered as examples for life on icy worlds. Indeed, these specialized phototrophic eukaryotes can colonize melting snow and ice, and high cellular abundances cause colourful blooms in mountainous and polar regions. This contribution depicts physiological and ecological capabilities of these microorganisms for tolerating low temperatures or harmful irradiation. Metabolic adaptations include rigid cell walls and protective intracellular pigmentation. Still, the life cycle comprises cell division as a critical moment, depending on the presence of liquid water. On the other hand, cyst-stages sustain freezing during winter and drought after complete melt during summer. Moreover, these microalgae were tested and proved to tolerate the harsh conditions of outer space for a certain period. Also, snow algae were successfully tested to generate biomass in an almost Martian atmosphere, thus representing potential organisms for food or feed production under low-pressure scenarios[1].

Finally, biosignatures for remote detection *in situ* are evaluated. Visible to near-IR Reflection-spectra[2] and Raman-spectra [3-5] of intracellular pigments (e.g. carotenoids) are candidates particularly convenient for such an approach.

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Thermoacidophilc verrucomicrobial methanotrophs thrive beyond the boundaries of methanotrophy

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Extremophilic verrucomicrobial methanotrophs living in acidic geothermal ecosystems were initially thought to be metabolically confined. Later, autotrophic growth on CO_2 with H_2 as an electron donor was observed in thermophilic and mesophilic verrucomicrobial methanotrophs. Here, we observed active propane consumption in *Methylacidiphilum*-dominated acidic geothermal samples incubated with methane+propane. Two pure cultures of *Methylacidiphilum* species (strains IT5 and IT6) derived from these enrichments, utilized oxygenated C3 metabolites of propane as sole carbon and energy sources, but not propane. The complete biochemical pathway for utilizing C3 substrates was predicted by genomic and transcriptomic analyses and supported by physiology and substrate specificity experiments. A gene cluster involved in the pathway of 2-propanol conversion to pyruvate via acetol was found and surprisingly, the *pmoCAB3*, one of the three operons encoding a copper membrane monooxygenase (CuMMO), resides in this cluster and is found to catalyze acetone oxidation to acetol. Coupling of PmoCAB1/2- dependent co-metabolic oxidation of propane to 2-propanol with a complete oxidation pathway of 2-propanol enabled complete oxidation of propane. Additionally, one of the isolates, strain IT6, respired a nitrogenous greenhouse gas, N_2O, as an alternative electron acceptor in anoxic conditions. Thus, the discovery of a novel function of CuMMO, the ability to utilize C3 compounds and respire an alternative electron acceptor expand the current metabolic traits of verrucomicrobial methanotrophs.

Photobiochemical response to high irradiance of an acidophilic microalga

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Coccomyxa onubensis is an extremophilic microalga that inhabits the acidic waters of the Tinto river and mine drainages in the Pyritic Belt area in the north of Huelva, Spain. The lower solubility of CO_2 and O_2 in the acidic waters limits the growth of photosynthetic species to the superficial layer of the acidic waters where carbon and oxygen availability are greater. Consequently, the photosynthetic apparatus of the acidophilic microalgae has to adapt to cope with the high irradiance levels of the region. This study was aimed at getting any insight into the microalgal response to increased levels of PAR irradiance, from 150 to 900 μmol photons·m^{-2}·s^{-1}, through the analysis of the photobiochemical performance. Among other parameters, the evolution of total chlorophyll content of the cells, the PSII efficiency and specific data related to the light absorption by the cells and its subsequent photobiochemical use were analyzed. The obtained data showed a significant reduction in the cellular chlorophyll pool size (by 50% at 900 μmol photons·m^{-2}·s^{-1}) as light irradiance increased, which can be a first sign of adaptation by reducing the number of light-harvesting membrane complexes. Nevertheless, the absorption flux per photosynthetic reaction centre (ABS/RC) at PSII increased by 27% in cells under 900 μmol photons·m^{-2}·s^{-1} compared to control cells, and the trapped energy flux per reaction centre (TR_0/RC) increased at the referred irradiance by roughly 15% while the electron transport flux (ET_0/RC) remained stable. The results allow us to suggest that *Coccomyxa onubensis* minimizes excess light damage effects by reducing the number of light capturing molecules and inactivating part of the reaction centres in order to limit the photon flux to be transduced through the photochemical reaction chain. This strategy would attempt to prevent the primary quinone acceptors pool to become fully reduced, then ensuring the activity of the electron flux chain from PSII to the final electron acceptors.

Acknowledgements This study was funded by Grant P20_00930 from the Andalusian Plan for Research, Development and Innovation, within the frame of the operational programme “FEDER Andalucía 2014-2020”.

Energy conservation by using dimethyl sulfoxide as alternative electron acceptor in the acetogen *Moorella thermoacetica*

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Acetogenic bacteria are an ecophysiologically important group of strictly anaerobic bacteria. Their characteristic feature is to oxidize organic as well as inorganic electron donors, coupled to the reduction of CO_2 via the Wood-Ljungdahl pathway as terminal electron acceptor. In addition to CO_2, acetogens can use alternative electron acceptors such as nitrate, fumarate, dimethyl sulfoxide (DMSO) or aromatic acrylates[1]. Astonishingly, little is still known about the bioenergetics of CO_2 reduction and the possible use of electron acceptors other than CO_2 in *Moorella thermoacetica*. By far the best studied alternative terminal electron acceptor is nitrate[2][3]. Here we have analysed whether or not *M. thermoacetica* can reduce DMSO as final electron acceptor. Growth of *M. thermoacetica* on glucose or H_2 + CO_2 was stimulated by DMSO. Membranes had a DMSO reductase activity that was induced by growing cells in presence of DMSO. The enzyme used reduced anthraquinone-2,6-disulfonate, benzyl- and methyl viologen as electron donor, but not NAD(P)H. Activity was highest at pH 5 and 60°C, the Km for DMSO was 2.4 mM. Potential DMSO reductase subunits were identified by peptide mass fingerprinting; they are encoded in a genomic region that contains three potential *dmsA* genes, three *dmsB* genes and one *dmsC* gene. Transcriptome analysis revealed that two different *dmsAB* gene clusters were induced in the presence of DMSO. In sum, the data are in line with the hypothesis that *M. thermoacetica* can use DMSO alongside CO_2 as electron acceptor and DMSO reduction is catalysed by an energy-conserving, membrane-bound electron transport chain with DMSO as final electron acceptor.

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Periplasmic surface immobilization of thermostable carbonic anhydrase

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Reactive absorption into aqueous solutions promoted by carbonic anhydrase (CA, E.C. 4.2.1.1.) has been developed as a CO_2 capture process [1]. Efficient biocatalysts based on carbonic anhydrase are based on covalent immobilization through several techniques including attachment on solids, cross-linking and matrix entrapment [2]. The present study is focused on the use of a thermostable carbonic anhydrase from *Sulfurihydrogenibium yellowstonense*. The enzyme has been produced as recombinant membrane-anchored protein in *Escherichia coli* cells [3] using the Ice Nucleation Protein of *Pseudomonas syringae* (INPN) as anchoring protein. The membrane debris recovered after cell harvesting, lysis, and membrane fractionation enzyme were used as characterized in terms of the enzyme (INPN-SspCA) loading and activity. The INPN-SspCA was present at about 1-2 mg/g. Moreover, the apparent kinetics of the biocatalyst was characterized through CO_2 absorption tests in a stirred cell lab-scale reactor assuming a pseudo-homogeneous behavior of the biocatalyst in the form of dispersed membrane debris. k_{cat}/K_M values up to 0.55 $L \cdot mg^{-1} \cdot s^{-1}$ were assessed at 25°C. The absence of enzyme leaching in the alkaline solvent was observed after 24 h and the equilibration of dispersed cell membrane debris in the alkaline buffer positively affected the performances of the heterogeneous biocatalyst by promoting the exposure of fine debris surface and thus of the anchored carbonic anhydrase. Future studies will focus on thermal resistance and activity of INPN-SspCA as well as on possible applications for CO_2 utilization in the aqueous phase.

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Diversity and hydrolytic potential of marine bacteria from the black sea

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Marine microorganisms represent promising sources of enzymes for various industrial and environmental applications that require harsh physicochemical conditions[1]. Considering the increasing demand on the global market for such biocatalysts, bioprospecting of under-investigated environments is essential to identify novel and more efficient producers of enzymes with unique catalytic properties. The current study explored the diversity of marine bacteria in seawater samples collected from two locations of the Romanian Black Sea seashore (i.e., Eforie Nord and Cap Aurora) and their potential to synthesize valuable hydrolytic enzymes for biotechnologies. Marine bacteria were obtained by culture-based approaches and identified based on 16S rRNA gene sequencing. A total of 64 halotolerant, slightly-halophilic and moderately-halophilic strains belonging to four phyla (*Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*) were isolated from the collected samples. Among them, 53 (83%) produced at least one of the six extracellular hydrolases tested, counting lipases (60.9%), amylases (43.7%), proteases (42.2%), cellulases (31.2%), pectinases (21.9%) and xylanases (17.2%). Bacterial isolates with the most promising catalytic activities belonged to genera *Pseudoalteromonas*, *Paraglaciecola*, *Polaribacter*, *Aquimarina*, *Cellulophaga*, *Bacillus*, *Metabacillus*, *Isoptericola* and *Streptomyces*.

Funding: ERANET-MARTERA-MOBILTOX-2

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Identification of hydrolytic enzymes by *in silico* analysis of the metagenome of the geothermal field "Los Humeros"

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In Mexico, there are few studies regarding bacterial diversity in environments with extreme conditions. The extremophilic microorganisms that inhabit these areas are a source of new enzymes and metabolites with interesting biotechnological and industrial potential. Independent culture techniques, such as metagenomic analysis, have enabled the study of new microorganisms, genes, metabolic pathways, and the characterization of the phylogenetic and functional composition of these microbial communities.

Los Humeros geothermal field is located at the eastern end of the Trans Mexican Volcanic Belt and possess steams soils with temperatures of 50-90ºC. By means of a whole-genome shotgun approach we obtained a metagenomic bank after the enrichment of a steam soil sample (15 cm depth) in a safflower oil rich medium [1]. The analysis revealed 165 genes encoding lipolytic enzymes, 43% of them uncharacterized. PHB is a bacterial polyester that thanks to its biodegradable qualities has attracted industrial attention as an ecological alternative to the use of plastics derived from petroleum to be used for a wide range of agricultural, marine, and medical applications [2]. PHB is degraded by a PHB depolymerase [3]. After analyzing the metagenomic sequences in different databases, we found a gene from *Thermogemmatispora argillosa* that encodes for a probable new PHB depolymerase. This new enzyme presents a 80 to 95% similarity with other PHB depolymerases reported in the NCBI database. The cloning, expression and characterization of the putative PHB depolymerase are currently being carried out at the lab.

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Functional analysis of *deinococcus grandis* ppri using reporter assay

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The genus *Deinococcus* is one of the microorganisms extremely resistant to radiation. The radioresistance of *Deinococcus* depends on its excellent ability to repair DNA double-stand breaks. In previous studies, several deinococcal DNA repair genes have been found to be induced by exposure to ionizing radiation[1]. These genes are repressed by a repressor protein DdrO, which binds to an operator sequence called RDRM (radiation/desiccation response motif). Following irradiation, DdrO cleaved by PprI no longer binds to RDRM, thereby, the expression of RDRM regulon is derepressed[2]. Thus, PprI serves as an activator for radiation-induced up-regulation of DNA repair genes in *Deinococcus* spp. PprI possesses three structural domains: zinc peptidase domain, helix-turn-helix DNA binding domain, and GAF-like domain[3]. However, the mechanism of the functional expression of PprI is still not clear. This study aimed to identify amino acid residues important for the functional expression of PprI in *Deinococcus grandis*.

A PprI deficient strain was generated using *D. grandis* TY3 as a parental strain. The strain was then transformed using two plasmids; a reporter plasmid carrying a luciferase gene under the control of the promoter and operator for the *D. grandis ddrO* gene, and a complementary plasmid carrying the *D. grandis pprI* with wild-type or mutant-type sequence. The changes in chemiluminescence signal of the transformants was monitored using a Dual-Glo luciferase assay following UV-C irradiation. As a result, glutamic acid 84 and histidine 87 in the zinc peptidase domain are critical for functional expression of PprI, consistent with a previous study obtained using *D. deserti.* It has been shown that a *D. deserti* mutant PprI protein designed to disrupt the protease activity sensitizes the cells towards UV-C[3]. On the other hand, the *D. grandis carrying pprI* with mutation-type sequence in HTH domain or GAF-like domain showed partial complementation, indicating these domains play some role in the functional expression of PprI.

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Exploration of iron reducing, thermoacidophilic archaea in Oku-Shiobara hot spring

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A-wide variety of thermoacidophilic archaea such thrive in volcanic acidic hot springs. As represented by the genera *Sulfolobus* and *Thermoplasma*, many of them are known to grow aerobically or anaerobically using sulfur or sulfur compounds.[1] On the other hand, some thermoacidophilic archaea have been reported to grow by oxidizing or reducing iron compounds.[2] However, it is not clear to what extent the iron metabolisms are distributed in Archaea. In our previous studies, representative strains of *Thermoplasmatales* and *Sulfolobales* grew anaerobically in the presence of Fe(Ⅲ). This finding would imply that many of thermoacidophilic archaea are capable of metabolizing iron compounds. On the other hand, our metagenomic analysis revealed that uncultivated archaea of *Thermoplasmatales* and *Sulfolobales* were predominant in the microbial communities of hot spring sites at Oku-Shiobara Arayu-Onsen, Tochigi, Japan. Therefore, we conducted anaerobic enrichment cultures in the presence of Fe(Ⅲ), for these samples, and successfully cultivated novel archaea belonging to *Thermoplsamatales*.

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Hunting for thermophilic plastic-active enzymes in a local wastewater treatment plant

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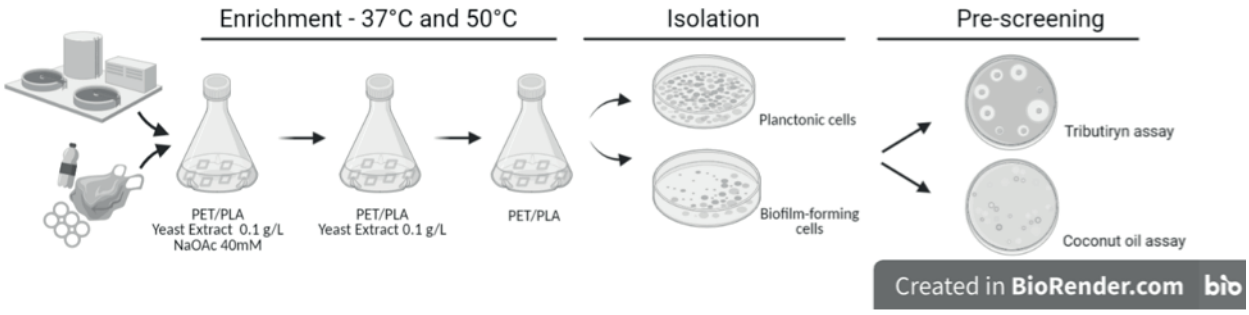
Plastic demand has drastically increased in the last decades, but little has been done to reduce its environmental impact. Most plastic waste is disposed of in landfills or dispersed, thus polluting terrestrial and marine environments[1]. Microbes thriving in these ecosystems may represent a reservoir of plastic-degrading enzymes. Therefore, we investigated the plastic-degradation potential of microbes isolated from a microplastic-abundant environment, i.e., a local municipal wastewater treatment plant. Cultivation media, containing post-consumer polyethylene terephthalate (PET) or polylactic acid (PLA), were used to favor the growth of mesophilic and thermophilic plastic-degrading microbes. In parallel, biofilm-forming and planktonic cells were sub-enriched on PET or PLA-emulsified agar plates[2]. Enriched consortia were screened on plates containing either short-chain (esterase-like enzyme activity) or middle-chain length triglycerides (lipase-like enzyme activity) to isolate producers of soluble plastic-degrading enzymes[3]. As a result, we have isolated several strains secreting esterase- and lipase-like enzymes, which are currently under investigation to assess their PET- and/or PLA-degradation activity via enzymatic assays[4], using plastics emulsions as substrates. Moreover, zymography, 3D fluorescence, and degradation assays will be performed to identify and characterize potential enzymes active on post-consumer plastic waste residues.



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Proteomic responses suggest the existence of proteins belonging to carbon fixation pathways in *carbonactinospora thermoautotrophica* StC

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Thermophilic bacteria are valuable sources of metabolic diversity and are currently being investigated for alternative carbon fixation pathways[1], mainly because a chemolithoautotrophic thermophile is probably the most common precursor to life and provides the best model for investigating primordial metabolisms[2]. The aim of this study was directed toward the label-free based quantitative proteomic analysis of *Carbonactinospora thermoautotrophica* strain StC, a thermophilic bacteria isolated from a Brazilian soil, aiming to estimate the proteins correlated with carbon fixation pathways. Here we used a linear relationship with the logarithm of protein concentration in LC-MS/MS to report the Exponentially Modified Protein Abundance Index (emPAI) values obtained in two different cultivation conditions: 1) input of CO, CO_2, and a mix of nitrogen and O_2 gasses; 2) input of CO, CO_2, and NH_4CL. Preliminary results of KEGG database showed the abundance of upregulated key enzymes belonging to five known natural carbon fixation pathways: Calvin cycle, Arnon-Buchanan cycle, 3-Hydroxypropionate bicycle, Hydroxypropionate-hydroxybutyrate cycle and Dicarboxylate-hydroxybutyrate pathway. The proteomic responses two different growth condition suggests of this thermophilic strain maybe can use one of these paths for fixing carbon from the atmosphere and utilize this carbon as energy source. Nonetheless, deeper analyses are ongoing with the proteomics data, as well as with the transcriptomics and biochemistry information to better elucidate the pathway that the strain StC uses for carbon fixation.

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The role of cell membrane on caffeine stress of *Lactobacillus paracasei*

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Caffeine, known as a component of coffee and energy drinks, has antibacterial activity against not only pathogenic bacteria but also against lactic acid bacteria (LAB) those are known as beneficial bacteria (1). Environmental stresses, such as pH, temperature, pressure, and chemical substance have various affect the growth, the morphology, and the fatty acid composition of their membranes of LAB (2, 3). However, there are few reports on the effects of caffeine on LAB.

We demonstrated that caffeine resistance with 2 isolates of caffeine-resistant LAB. These isolates were isolated from traditional foods and vegetables by appropriate dilutions with DDW, plating on MRS agar supplemented with 1% $CaCO_3$ to distinguish the acid-producing bacteria from other bacteria and then incubating anaerobically at 30 °C for 48 h. The caffeine-resistant LAB YSAK1 and YKP4 were identified as *Lactobacillus paracasei* using MALDI-TOF/MS. *L. paracasei* YSAK1 and YKP4 have shown clearly *resistance* to caffeine compared to the type strain *L. paracasei* NBRC 15889 ^{T}. YSAK1 and YKP4 had high Colony Forming Unit (CFU) at 1.0% caffeine. On the other hand, the CFU of *L. paracasei* NBRC 15889 ^{T} at caffeine concentrations of 0~1.0% decreased significantly from 0~0.3% and decreased slowly from 0.4~1.0%. Therefore, we focused on the cell membrane and conducted research. SEM observation after culturing in the presence of caffeine, only *L. paracasei* NBRC 15889 ^{T} showed elongation of the bacteria at 0.2%~1.0% and damage to the surface of the bacteria at 0.5%~1.0%. The proportions of saturated fatty acids in the type strain and unsaturated fatty acids in caffeine-resistant strains increased, respectively. The fatty acid composition of the type strain also changed, with an increase in the percentage of palmitic acid (C16:0) and a significant decrease in the percentage of dihydrosterculic acid (cyc-C19:0 (c9)) in the presence of caffeine.

These results suggest that caffeine may affect multiple mechanisms.

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Identification of a previously unrecognized nucleoside degradation pathway in haloarchaea

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Many archaea do not harbor the non-oxidative pentose phosphate pathway to metabolize the ribose moieties of nucleosides[1]. Instead, Thermococcales utilize a pentose bisphosphate pathway which is composed of three nucleoside phosphorylases, ADP-dependent ribose-1-phosphate (R1P) kinase, NMP phosphorylase, ribose-1,5-bisphosphate (R15P) isomerase, and ribulose-1,5-bisphosphate (RuBP) carboxylase/ oxygenase (Rubisco)[2-3]. The latter three enzymes, forming an NMP metabolic pathway, are widely distributed in Archaea. However, multiple halophilic archaea seem only to possess R15P isomerase. In some haloarchaea including *Halobacterium*, we identified a previously unrecognized nucleoside degradation pathway composed of guanosine phosphorylase, ATP-dependent R1P kinase, R15P isomerase, RuBP phosphatase, ribulose-1-phosphate (Ru1P) aldolase, and glycolaldehyde reductase. In the pathway, the ribose moiety of guanosine is converted to dihydroxyacetone phosphate (DHAP) and ethylene glycol via the route guanosine → R1P → R15P → RuBP → Ru1P → DHAP + glycolaldehyde → DHAP + ethylene glycol. Individual or coupling activities of all enzymes constituting the pathway could be detected in the cell-free extract of *Halobacterium salinarum*, suggesting the presence of the nucleoside degradation pathway in the organism. Although the metabolic route from nucleoside to RuBP via R15P is similar to that of the pentose bisphosphate pathway in Thermococcales, the downstream route does not involve Rubisco and is unique to halophilic archaea.

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Glycerol metabolism in *sulfolobus acidocaldarius* involves an unusual glycerol-3-phosphate dehydrogenase

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Glycerol is an integral constituent of membrane phospholipids in all domains of life and is thus a highly abundant organic compound in nature. Glycerol processing in bacteria is well studied and follows two possible biochemical routes converting glycerol into dihydroxyacetone-phosphate[1, 2]. Conversely, in the archaeal domain the glycerol metabolism has only been studied in the halophile *Haloferax volcanii* so far[3]. Here we show that glycerol utilization in the thermoacidophilic Crenarchaeon *S. acidocaldarius* proceeds via phosphorylation to glycerol-3-phosphate (G3P) and subsequent oxidation to dihydroxyacetone-phosphate catalyzed by the glycerol kinase (GK) and G3P dehydrogenase (G3PDH), respectively. Furthermore, a detailed characterization of the G3PDH (Saci_2032) is reported. The purified, recombinant G3PDH showed a homodimeric structure in solution, contained one non-covalently bound FAD cofactor per subunit and was stably reduced by G3P. The enzyme was specific for G3P (v_{max}: 44.5 U/mg; K_M: 0.055 mM) and transferred electrons to artificial acceptors dichlorophenolindophenol and ubiquinone-Q1, *in vitro*, indicating that caldariella quinone is the natural electron acceptor *in vivo*. Accordingly, the G3PDH activity, highly induced in glycerol grown cells, was partly associated with the membrane. This membrane association was shown to be essentially different compared to known G3PDHs and was mediated by a small protein encoded downstream of the *saci_2032* gene annotated as coxG homologue. Together with distinct sequence differences compared to known G3PDHs, this unique membrane anchoring suggests Saci_2032 as representative of an unusual type of G3PDH in *Sulfolobales*.

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Glycerol metabolism in Sulfolobus acidocaldarius: Characterization of two glycerol kinases

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Glycerol is an integral constituent of membrane phospholipids in all domains of life and also of storage lipids mainly known from bacteria and eukaryotes and is thus a highly abundant organic compound in nature. Accordingly, many organisms utilize glycerol as carbon and energy source. However, although the genetic capacity to grow on glycerol was reported for several archaea [[1]] detailed analyses of the glycerol degradation in this domain of life has so far only been reported for the halophile *Haloferax volcanii* [[2]]. In this study, the glycerol degradation in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* was studied. Results from transcriptomic, proteomic and metabolomic analyses as well as crude extract measurements indicated that *S. acidocaldarius* degrades glycerol via one of the two commonly known pathways, starting with the phosphorylation to glycerol-3-phosphate followed by oxidation to dihydroxyacetone phosphate (DHAP) which then enters the common lower shunt of the ED and EMP pathway. The reactions are catalyzed by glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively. However, the pathway exists in two paralogous copies in *S. acidocaldarius*. To get first insights into their distinct function the two glycerol kinases were homologously overproduced in *S. acidocaldarius*, purified and characterized. The molecular and kinetic properties are presented. Together with the regulatory pattern the data indicate that both paralogues might be involved in glycerol breakdown.

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Transformation of Leptolyngbya sp. NIES-2104 by Electroporation

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Leptolyngbya boryana 6306 and *Leptolyngbya* sp. NIES-2104 are cyanobacterial species found in fresh water and terrestrial habitats respectively. A comparative genomics study of the two species revealed genetic differences that likely confer to *Leptolyngbya* sp. NIES-2104 the ability to survive dry environmental conditions (1). Although comparative genomics provides a deeper understanding of desiccation adaptive mechanisms, DNA transformation systems can facilitate the development of complementary tools that can be used to further investigate and/or confirm proposed mechanisms. While a method for *L. boryana* electrotransformation has been established (2), a transformation system for its terrestrial relative has not. Cyanobacteria compatible plasmids pPMQAK1-tre and pAM1954 express a GFP linked trehalose biosensor and GFP alone respectively. Herein a method of transformation of *Leptolyngbya* sp. NIES-2104 by electroporation is described. Transformation was confirmed with PCR and fluorescence, providing evidence for the utility of pPMQAK1-tre and pAM1954 plasmid backbones in the development of a genetic system in this species.

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Prokaryotic diversity of tropical coastal sand dunes ecosystem using metagenomics

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Coastal sand dunes (CSDs), unique, stressed and hostile habitats act as a barrier between marine and terrestrial ecosystems. CSDs are stressed in terms of nutrition and fluctuating physio-chemical conditions. CSD is classified into several types, each of which presents different challenges for life forms. This study focuses on exploring bacterial and archaeal diversity and community structure in four CSD namely, Embryo, Fore, Gray, and Mature dunes of Keri beach, Goa along the west coast of India. The study was carried out using Next Generation Sequencing of hypervariable V3–V4 regions of the 16S rRNA gene using Illumina HiSeq platform. The present study hypothesizes that the prokaryotic communities at each dune may be different and could have different roles in the ecosystem. The NGS for Embryo, Fore, Gray, and Mature dunes gave 1,045,447, 1,451,753, 1,321,867, and 1,537,758 paired-end reads, respectively, out of which 54,500, 50,032, 37,819, and 111,186 were retained through various quality filtrations. A total of 74, 63, 65, and 65% of OTUs, respectively, remained unknown at the species level. The highest bacterial and archaeal abundance was reported from Mature and Embryo dunes, respectively. Phylum Actinobacteria dominated the Embryo, Fore, and Mature dunes, whereas Phylum Proteobacteria was dominant in the Gray dune. Streptomyces was predominant in overall CSD followed by Bacillus, Acidobacterium, and Kouleothrix. The commonly and exclusively found members in each dune are cataloged. The highest species dominance, diversity, species richness, and abundance were observed in Embryo, Fore, Gray, and Mature dunes, respectively. The present study clearly elucidates that each dune has a distinct microbial community structure.

New Anti-inflammatory Compounds from the Deep-Sea Fungus *Cystobasidium laryngis*

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As demonstrated by Brimble's review, extremophiles are rich source of novel secondary metabolites. However, when compared to the large number of extremophilic microorganisms which have been reported, very few have been screened for the production of interesting secondary metabolites[1]. In our continuing search for bioactive compounds from extremophiles, we encountered a rare marine-derived yeast-like fungus *Cystobasidium laryngis* isolated from a deep-sea sediment sample collected from the Indian Ocean Ridge at the depth of 4,317 m in 2017[2]. From this deep-sea derived fungus, we could isolate three new anti-inflammatory metabolites. The structures of the new compounds were determined by analysis of spectroscopic data, semi-synthesis and comparison of optical rotation values. These compounds showed nitric oxide (NO) production inhibitory effect against lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cells without cytotoxicity at concentrations up to 30 μg/mL.

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Selection of potential PET hydrolases from a metagenome of geothermal origin through *in silico* probes

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Hydrolases are the most popular enzymes used in industry; of special interest are those obtained from thermophilic microorganisms. Although there is a great microbial diversity in extreme environments, there are limited studies intended to detect and isolate enzymes with potential in the degradation of polyethylene terephthalate (PET) [1]. Hydrolases for PET degradation are classified as carboxylic ester hydrolases (EC 3.1.1) [2]. Los Humeros geothermal field is located in Puebla, México, with surface temperatures between 50 - 90 °C [3]. A metagenomic bank was obtained by whole-genome shotgun after enrichment of a steam soil sample (15 cm depth) in a safflower oil rich medium. 106,660 contigs corresponding to protein domains were obtained, and a selection method was implemented through *in silico* probes designed to find genes encoding PET hydrolases, based on three sites of the primary structure of PET hydrolases with confirmed activity and reported structural data, corresponding to the nucleophilic elbow, the oxyanion hole and the histidine-fenced region of the catalytic triad. The probe based on the nucleophilic elbow sequence allowed the selection of six contigs that revealed active site homology to PET hydrolases with activity on PET film. The cloning, expression and characterization of the selected genes are currently being carried out at the lab, which will corroborate the effectiveness of the probes designed *in silico* for PET hydrolases screening.

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analysis of two aminotransferases from *Thermococcus kodakarensis* KOD1

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Pathways for amino acid utilization are important components of central carbon and nitrogen metabolism. Aminotransferases play a key role in carbon and nitrogen metabolism and have attracted much attention. On the genome of *Thermococcus kodakarensis*, there are more than 20 genes whose products display homology to previously identified aminotransferases. A number of those proteins have been characterized and the results show that TK2101 encodes an ornithine ω-aminotransferase involved in proline biosynthesis[1] and that TK1211 encodes an amino acid racemase with specificity towards leucine and methionine[2]. Here, we examined the activity and function of two aminotransferase homologs TK0548 and TK2268, which are annotated as aromatic aminotransferase (AroATh) and aspartate aminotransferase (AspATh), respectively. The two proteins were purified to apparent homogeneity by heat treatment or nickel chelate affinity chromatography followed by anion-exchange chromatography and gel filtration chromatography. The assembly analyses of these two proteins revealed that the molecular mass of the TK0548 protein is about 80 kDa, suggesting that the protein forms a dimer, whereas in the case of the TK2268 protein, the molecular mass was estimated to be about 300 kDa, much larger than most other previously studied aspartate aminotransferases. Activity measurements revealed that the TK0548 protein displayed relatively high aminotransferase activity when aromatic amino acids were used as amino donor with 2-oxoglutarate as the amino acceptor. The TK2268 protein displayed relatively high activity toward glutamate and aspartate when pyruvate was used as the amino acceptor. The different preferences towards amino acids suggest their contribution to amino acid assimilation in this organism.

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The effects of temperature and perchlorates on *Deinococcus radiodurans*' metabolic activity

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The extremophile bacterium *Deinococcus radiodurans* is characterized by its ability to survive and sustain its activity at high levels of radiation and is considered one of the organisms that might survive in extraterrestrial environments. In the present work, we studied the combined effect on *D.radiodura*ns' CO_2 production rates of temperature and perchlorate salts, the latter of which have been previously detected in the surface regolith of Mars. When tested under different incubation temperatures with constant perchlorate concentration (1\% w/v), metabolic activity was found to depend mainly on the temperature while the specific perchlorate salt concentration used did not seem to affect the activity greatly. Reduced CO_2 production capacity was observed when testing the metabolic activity at higher perchlorate concentrations (2.5, 5 and 10% w/v) at different incubation temperatures varying between 0 and 25°C. The metabolic activity was reduced as the perchlorate concentration increased and temperature decreased, but no interactive effects of temperature and perchlorate concentration on the metabolic activity was found. These results might be indicative of *Deinococcus radiodurans*' ability to remain metabolically active in environments rich in perchlorate and with low temperature such as the Martian surface.

Thermal radon springs as a source of interesting microorganisms

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Mineral waters are an environment in which microorganisms with specific properties occur. The specifics of the environment force these microorganisms to develop unique metabolic pathways that can be used, for example, in bioremediation technologies or in the search for substances with high biological activity. Microorganisms from extreme environments have these properties and can therefore be used in biotechnology[1,2]. Bacterial communities from radioactive mineral water could be a promising source of microorganisms with extraordinary properties. The mineral springs in Jáchymov excel in their high concentration of dissolved radon. The radioactivity of the springs reaches 24 kBq/L[3]. In this work, bacterial isolates obtained from 4 springs from Jáchymov, Czechia, were investigated. A total of 6 most resistant isolates were selected from dozens of isolates during screening. All selected isolates form colonies coloured yellow, pink, or red on solid soil. Taxonomic identification was performed using 16S rRNA sequencing. Bacterial strains belonging to the genera *Kocuria, Rothia, Gordonia* and *Rhodococcus* were identified among the examined isolates. The isolates were investigated for resistance to stress conditions, which include ionizing radiation (gamma radiation and UV-C), the presence of free radicals (H_2O_2) or genotoxic substances (mitomycin) in the culture medium, and long-term exposure to low water activity. Isolates 101 and 201 (*Kocuria* sp.) excel in high resistance to ionizing radiation. Isolates 214 (*Rothia* sp.) and 215 (*Dietzia* sp.) showed moderate resistance to ionizing radiation, but at the same time high resistance to the presence of peroxide and mitomycin. Isolate 308 (*Rhodococcus* sp) had the highest resistance to long-term desiccation. Test results suggest that isolates from radioactive sources could have mechanisms in place to help them survive in highly extreme conditions and could be used in biotechnological processes.

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Microbiome of the northernmost mediterranean salterns (sečovlje salterns, slovenia)

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Solar salterns consist of a series of basins in which seawater is gradually concentrated until precipitation of table salt or halite, the main product of the activity, occurs. After the salt is harvested, the salt pans remain with bittern, which until recently was considered an aseptic environment due to its high concentration of magnesium salts. The aim of our study was to describe the microbial diversity (archaea, bacteria, fungi and other eukaryotes) in brines of different concentrations and in bittern from the Sečovlje salterns (Slovenia). We used a combination of metagenomics (amplicon sequencing) and culture-based methods to study the saltern microbiota.

From brines of different salinity, we isolated bacterial strains belonging to the genera *Bacillus*, *Chromohalobacter*, *Cobetia*, *Halomonas*, *Larsenimonas*, *Salicola*, *Salinicola*, and *Staphylococcus*, as well as archaea from the genera *Halococcus*, *Halorubrum*, and *Halovivax*. Using amplicon sequencing of the 3rd and 4th variable regions of 16S rDNA, we discovered that the predominant bacterial genera in the brine and bittern of the crystallization ponds were *Salinibacter* and *Salinivenus*, while the predominant archaeal genus was *Halorubrum*. The majority of fungal isolates from the brine and bittern belonged to Ascomycota, from genera *Cladosporium*, *Aspergillus*, and *Penicillium*; a few were basidiomycetes, mostly from genus *Wallemia*. Black yeast-like fungi (Dothideales) and non-melanized asco- and basidiomycete yeasts were also present. Amplicon sequencing of the ITS2 region allowed us to detect 53 different fungal genera. Using 18S rDNA amplicon analysis, we also identified other eukaryotes, primarily ciliates from the genera *Fabrea* and *Eutintinnus*, from Myzozoa genus *Eudubosquella*, and green algae from the order Chlamydomonadales.

With increasing salinity, the alpha diversity of bacteria, archaea and other eukaryotes decreased, while the alpha diversity of fungi was the lowest at moderate salinity (10 - 20 °Bé).

The Biorecover 2020 project for the recovery of critical raw materials and rare earth elements from waste ores

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Industrial wastes generated during processing of mineral ores present not only a health and ecological hazard, but also an exploitable resource. Aluminium and, magnesium residues and end-of-life rare earth element-containing (REE) waste products are frequently resistant to bioleaching as result of high alkalinity, chemical toxicity, or by the enrichment of radioactive elements. Technical and economic barriers to the processing of some waste ores have resulted in extensive stockpiling. If technologies were developed to extract elements from these waste ores they would represent valuable secondary streams of rare earth elements (REEs), such as scandium, and critical raw materials (CRMs). Global bauxite residue (red mud) stockpiles alone account for 4 billion metric tons of waste ore residue, with an annual contribution of 175.5 million tons, and is estimated to contain 480 000-600 000 metric tons of scandium. The EU-funded Biorecover 2020 project investigated the bioleaching of several waste ore streams with a focus on leach efficiency, early process design, and downstream processing. Several organic and mineral acids were tested in shake flask experiments to inform leaching strategies, followed by contact leaching experiments with biogenic acids to solubilise the target metals in shake flasks and bioreactors. The data point towards promising strategies for the recovery of target metals at industrially relevant rates and purities. Future work will involve the optimisation of leach rates followed by scale-up of the process to evaluate it for potential industrial implementation.

Keywords: leaching, mineral acid, organic acid, rare earth elements, waste ore

Acknowledgements: This study was funded through the EU Horizon 2020 research & innovation program Biorecover project (Grant 821096).

Elucidating Mechanisms of Iron and Sulfur Oxidation in Thermoacidophiles

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The archaeal order Sulfolobales thrives in hot acid by conserving energy from oxidation of iron sulfide ores in order to power CO_2 fixation. Recently, interest in these organisms has peeked due to their bioleaching potential as global demand for copper, nickel, and other ore-bound metals increases[1]. However, the means by which the Sulfolobales access these substrates are still somewhat unclear. Here, we examine the underlying mechanisms involved in iron and sulfur oxidation by species in the Sulfolobales through the lens of comparative genomics[2], thermodynamics of both enzymatic and abiotic reactions[3], and surface interactions through biofilms. Key genes related to iron and sulfur oxidation arising from these analyses are further examined through protein characterization[4] and engineering of key genes into the obligate heterotroph *Sulfolobus acidocaldarius* to enable it to oxidize inorganic substrates[3,5]. Biofilms appear to be key to bioleaching and biofilm formation is examined for key thermoacidophiles. Finally, these mechanistic insights are used to probe differences among thermoacidophiles for extracting copper from chalcopyrite ores.

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Characterization of a thermostable red fluorescent protein variant DsRmCh and its heterologous expression in the thermophilic archaeon *Sulfolobus acidocaldarius*

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Fluorescent proteins are typically used as a reporter tool in various host organisms, for example for visualization or gene expression monitoring, but are not yet widely available for use in thermophilic archaea. More specifically, a fluorescent protein that is functional for gene reporter assays has not yet been described for *Sulfolobus acidocaldarius*, which is considered as a model species for thermoacidophilic Crenarchaeota. Therefore, the main aim of this work is the development of a fluorescent protein that is functional in *S. acidocaldarius* and can be used as a novel research tool. Using a pRN1-based shuttle vector system, we have analyzed the heterologous expression of several previously described thermostable green fluorescent protein variants (sfGFP, TGP, YFP) in *S. acidocaldarius*. None of them resulted in the observation of a significant fluorescence signal, although this might be due to the high background fluorescence emitted by the cells and medium in the green spectrum range (485–510 nm). For this reason, we next propose to test a red fluorescent protein variant, named DsRmCh, which is a fusion protein between DsRed and mCherry, and was previously demonstrated to have a good thermal stability and brightness at high temperature (Wannier, Moore et al. 2015).

We firstly characterized DsRmCh *in vitro* by heterologously expressing the protein in *Escherichia coli* followed by Ni-NTA affinity chromatography. The purified protein was subjected to fluorescence assays at different temperatures, in varied buffer conditions and at different wavelengths. This demonstrated that DsRmCh retains about 50% and 16% of its initial fluorescence intensity upon incubating during 17 hours at 75°C or 99°C, respectively. Besides, tests performed at different pHs indicated that the activity of DsRmCh remains essentially constant between pH 3.6 and 9.0. Next, DsRmCh was heterologously expressed in *S. acidocaldarius* by utilizing a pRN1-based *Sulfolobus*-*E. coli* shuttle vector, followed by fluorescence measurements. Furthermore, we successfully transformed the constructed shuttle plasmid pRN1-DsRmCh into *S. acidocaldarius* SK1 and induced expression by employing a maltose-inducible promoter, however, fluorescence was not detected. Therefore, we are currently performing qPCR analysis of the plasmid copy number to investigate whether a lack of fluorescence might be due to the stability of shuttle vector itself. In conclusion, DsRmCh exhibits good thermal stability and might be a good candidate to use as a reporter tool in *S. acidocaldarius*. However, possibly the shuttle vector plasmid is not always stably retained upon expressing the fluorescent protein.

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Functional dissection of structural regions of the *thermus thermophilus* competence protein pilw: implication in secretin complex stability, natural transformation and pilus functions

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DNA transfer via natural transformation is suggested to play a major role in adaptation to different environments. The thermophilic bacterium *Thermus thermophilus* exhibits the highest natural transformation frequencies known to date and is suggested to play a major role in DNA transfer in hot environments. Analyses of this DNA transporter unravelled a macromolecular transport machinery of 16 subunits spanning the cell periphery [1]. One subunit of the DNA transporter, the secretin PilQ, was found to form dynamic outer membrane (OM) channels playing a dual role in pilus extrusion and DNA uptake. The localization of PilQ in the OM was strictly dependent on the unique PilW protein which is essential for natural transformation and piliation [2]. Analyses of the interaction of PilW and PilQ revealed that PilW and the secretin PilQ form high molecular mass complexes in the OM. In a Δ*pilW* deletion mutant only PilQ monomers were detected. This suggests that PilW is important for PilQ complex assembly or stability. PilW comprises an intrinsically disordered region, whose deletion ($pilW_{\Delta 163\text{-}216}$) led to impaired natural transformation and abolished type IV pili mediated twitching motility [3]. These mutant phenotypes suggest that the disordered region in PilW induces conformational changes in the PilQ complex affecting pilus dynamics and DNA uptake.

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Archives and museum repositories are habitats for xerophilic fungi

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Ambient conditions in archives and museum repositories such as temperature and moisture are regulated for preventing microbial contamination of stored objects and their subsequent deterioration. Nevertheless, fungi known for their xerophilic nature are commonly encountered contaminants today. We discovered *Aspergillus halophilicus,* one of the most xerophilic fungi known, in an archive on book covers, concrete walls, and iron cassettes. Massive growth was encountered especially on concrete walls associated with fox-like colour stains, a phenomenon called foxing that is otherwise known from old paper.

The measured ambient temperature and relative humidity (RH) in the archive on the day of sampling was 7.5 °C and 40% RH. Most isolates recovered on malt/yeast extract agar with 50% glucose by dry swabbing from various sampled surfaces were identified as *Aspergillus halophilicus,* also recognised by it's morphology on adhesive tape samples taken directly from mouldy surfaces. Additional accompying fungi were identified as xerophilic *A. vitricola* and several other xerotolerant species including *A. montevidensis*, *A. jensenii*, *A. pseudoglaucus*, *Cladosporium halotolerans*, and *C. neolangeronii*. In addition to culturing, amplicon sequencing of the ITS2 region and the 3rd and 4th variable regions of 16S rDNA was used to determine the diversity of culturable and non-culturable fungi and bacteria in the archive.

Aspergillus halophilicus is occasionally reported from library materials and paintings but media used for its detection must have a maximum water activity of a_w = 0.89 to allow its growth. As it only develops on media sufficiently enriched with sugar, NaCl, and glycerol or a combination of these solutes it is possible that its occurrence in indoor environments is underestimated.

The growth of *A. halophilicus* on concrete walls, visible as foxing, is a new discovery. Its degradation capacity, currently studied on the level of cultures and genome, will be presented.

Expanding the genetic toolkit for *T. kivui*: identifying sugar inducible and repressible promoters in a model thermophilic acetogen

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Thermoanaerobacter kivui is a thermophlic (Topt = 66°C) acetogen capable of growth on H_2/CO_2 and simple sugars without the need for added vitamins[1]. The recent development of a genetic system[2], coupled with its robust autotrophic growth in minimal media, makes *T. kivui* an excellent candidate for industrial biotechnology applications[3]. However, thus far all recombinant protein expression in *T. kivui* has been carried out using just a few constitutive promoters. Inducible promoters would allow for finer control, for example for metabolic engineering or the production of toxic compounds.

The best characterized inducible promoters are sugar-induced, such as the Lac-operon in *E. coli*, and sugar inducible promoters have been developed for several model thermophiles[4, 5]. *T. kivui* is capable of growth on only four sugars (glucose, fructose, mannose, and mannitol), each of which appears to be imported by a dedicated phosphotransferase (PTS) system. We determined that *T. kivui* carefully regulates these uptake systems in response to available sugars. The cells treat mannitol as a less preferred substrate, repressing expression of the mannitol PTS genes in the presence of other sugars. This is in contrast to the fructose-specific PTS operon, which is strongly induced by the presence of fructose even when other sugar substrates are available. The fructose promoter also shows weak induction by mannose, allowing the same promoter to be used for either high or intermediate protein expression.

The two sugar inducible promoters identified here will allow for finer control of recombinant protein expression and metabolic engineering in T. kivui. However, the most promising biotechnological aspect of T. kivui is its ability to fix CO_2 under autotrophic conditions. Therefore, next steps include identification of promoters that can be induced without the need for addition of a heterotrophic growth substrate.

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Utilization of processed biomass by terrestrial deep subsurface microorganisms

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The diversity of microbial life present in the terrestrial deep subsurface is not fully understood. High temperature, high pressure, and a paucity of oxygen and nutrients make the terrestrial deep subsurface an extreme environment, yet certain microorganisms are able to reside therein. Microorganisms from a depth of 750 meters in the BLM-1 borehole were successfully enriched with biomass processed by Oxidative Hydrothermal Dissolution as a primary carbon and energy source. Analysis of enrichment bioreactor flow-through by High Performance Liquid Chromatography revealed utilization and conversion of carbon molecules in the processed biomass by the microbial community. Next generation sequencing of the 16S rRNA gene provided a profile of unique members of the microbial community able to utilize the carbon source.

Effect of Strong Salinity Stress on *Phaeocystis antarctica* and *Chaetoceros* spp. from the Ross Sea (Antarctica)

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Coastal Antarctic pelagic food webs are primarily based on two main photoautotrophic functional groups: diatoms and haptophytes (e.g., *Chaetoceros* spp. and *Phaeocystis antarctica*). The relative dominance the two groups varies on different temporal and spatial scales, thereby affecting trophodynamics and CO2 drawdown processes in the Ross Sea. Organisms in the Ross Sea must cope with multiple environmental perturbations, as shift in salinity driven by melting processes or sea-ice formation. In recent years, a refreshening of Ross Sea water have been reported in relation with ongoing climate changes, although there is still poor information on its effect on main phytoplankton species of pelagic food web. In the present study, we investigated the effect of salinity stress on *P. antarctica* and *Chaetoceros* spp. grown at salinity 20 (intense freshwater input), 34 (control), and 60 (brine channels). For all treatments, nutrient uptake, growth-rate and photosynthetic activity have been measured for 15 days in order to characterize the response of the two species under different saline conditions. Results indicate a different response of the two species contrasting rapid salinity changes, with different photosynthetic plasticity and relatively higher values of Fv/Fm at salinity 20 compared to 60.

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