The GOLLUM PROJECT

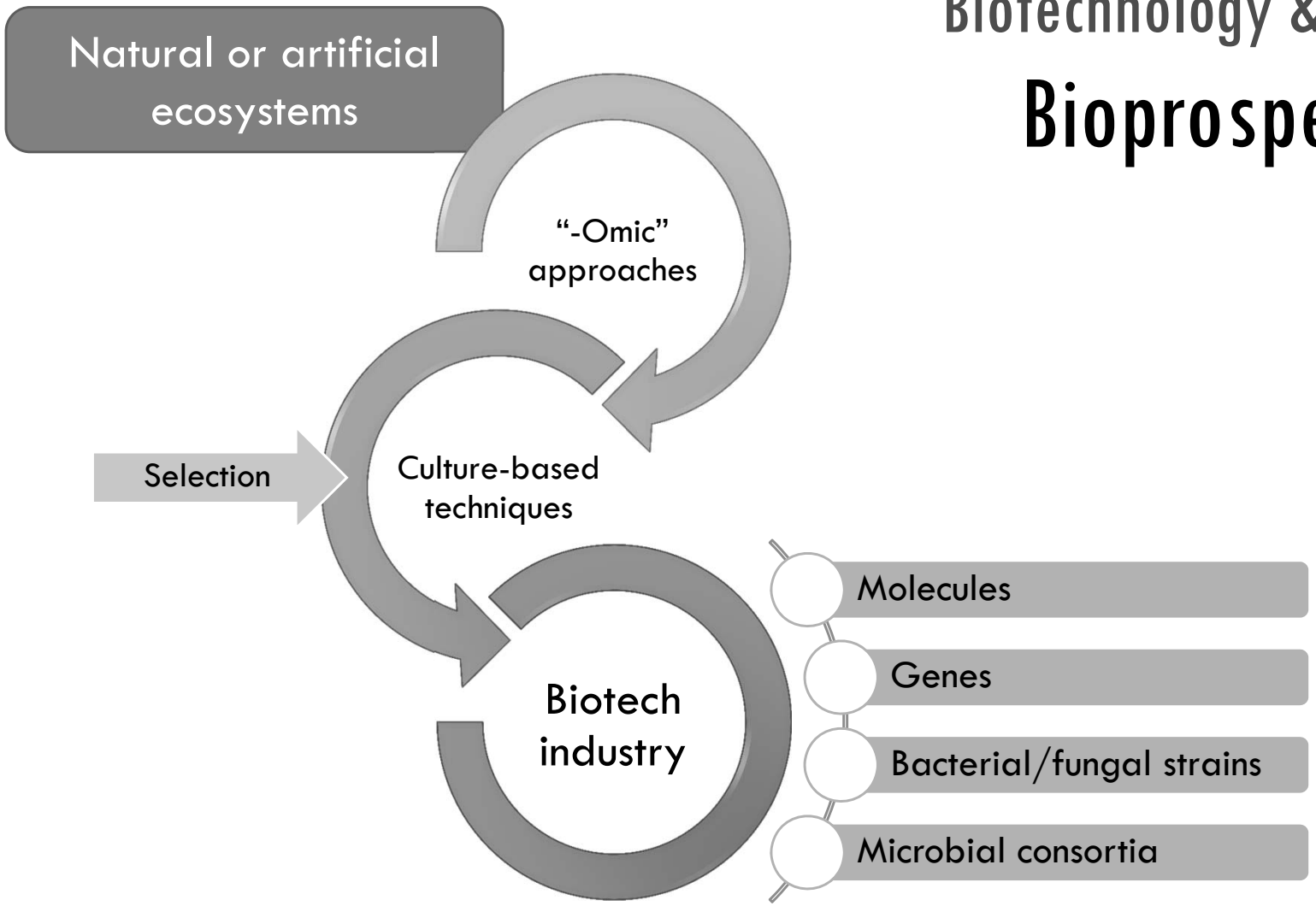
Characterising subterranean bacterial communities in depth(s)

Cristina Vilanova

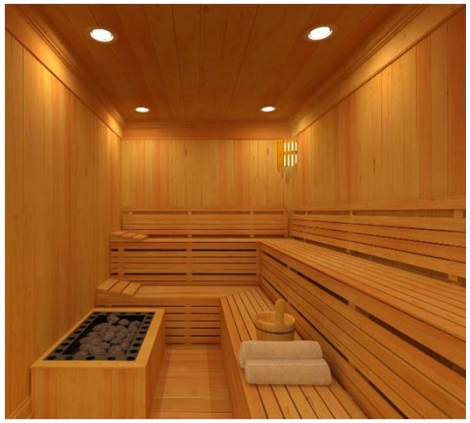
Biotechnology & Synthetic Biology Lab
Universitat de València

Biotechnology & SB Lab

Bioprospection



Bioprospection of “extreme environments”

Natural	Artificial		
Toxic-rich substrates	High temperature	High concentration of toxics	High UV radiation (and more)
			

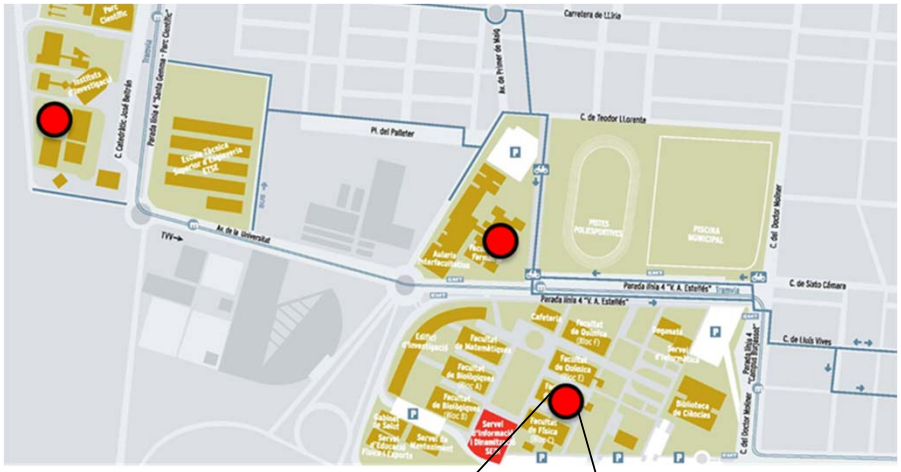
SOLAR PANELS: UNIC (AND YET UNEXPLORED) BIOTOPES

- Smooth flat surface
- Minimum water retention capability
- Maximum sunlight exposure

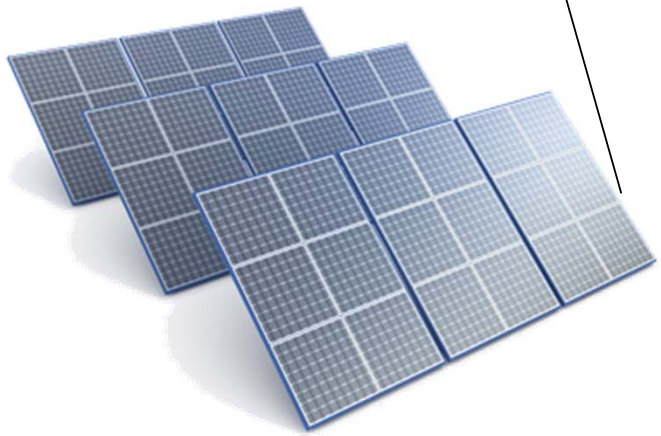
Circadian and annual peaks of:

- Desiccation
- Heat
- UV irradiation → **Carotenoids**

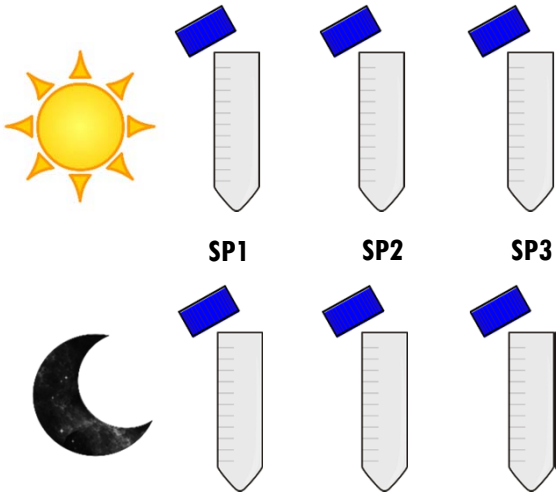
SAMPLING PROCESS & EXPERIMENTAL APPROACH



Summer solstice 2013



Summer solstice 2014



In vitro cultivation & characterization

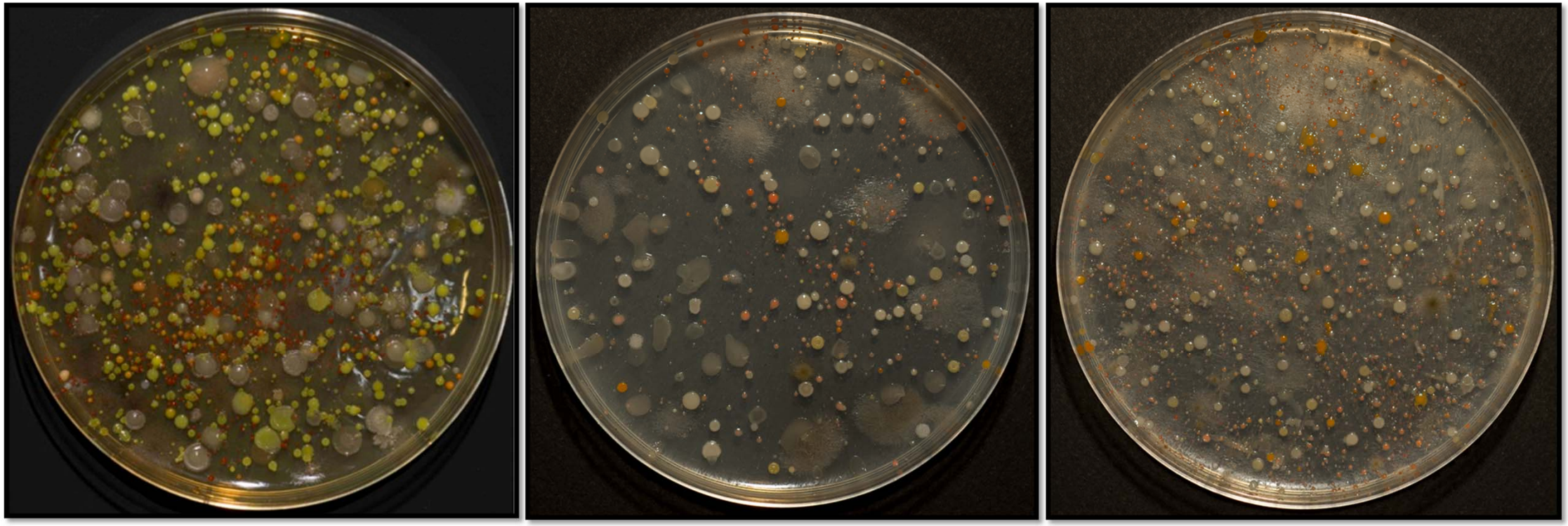
16S/18S sequencing

Shotgun metagenomic sequencing

Metaproteomics

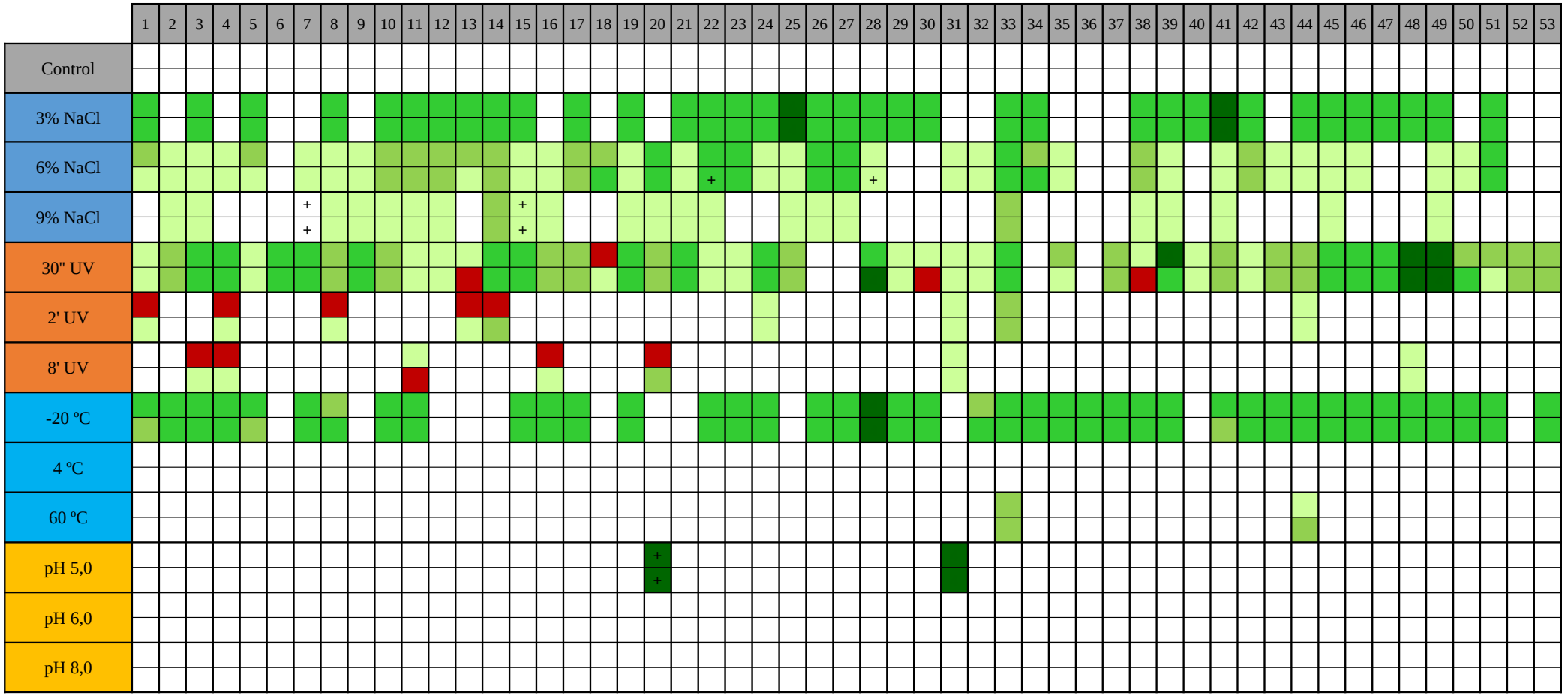
IN VITRO CHARACTERIZATION OF SOLAR PANELS' MICROBIOME

- Pigment (carotenoids) production

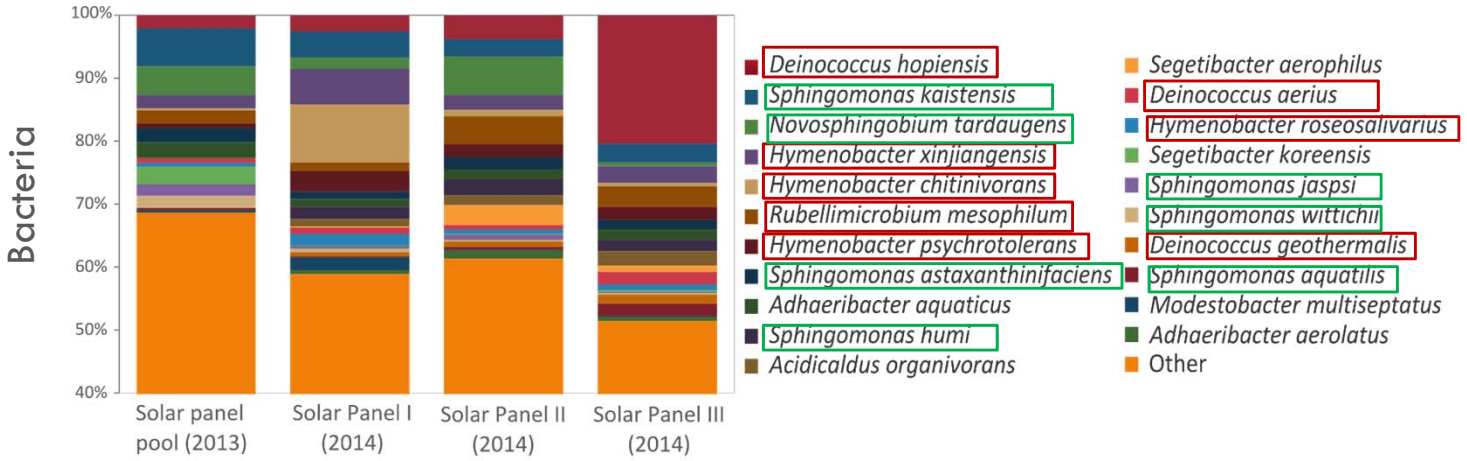


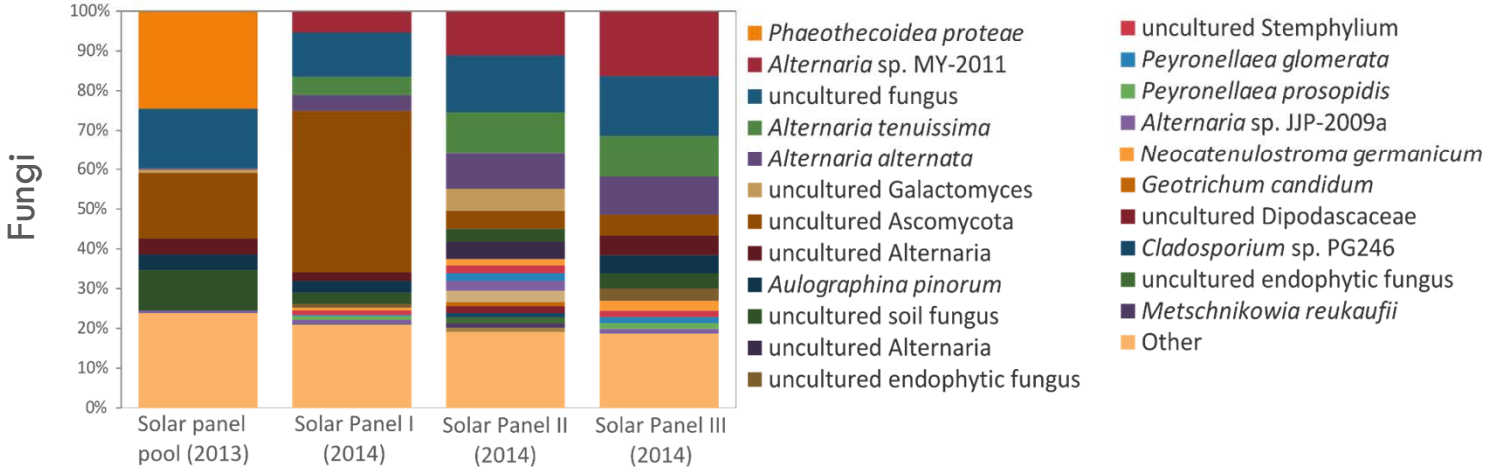
IN VITRO CHARACTERIZATION OF SOLAR PANELS' MICROBIOME

- Stress tolerance tests



METAGENOMIC PROFILE

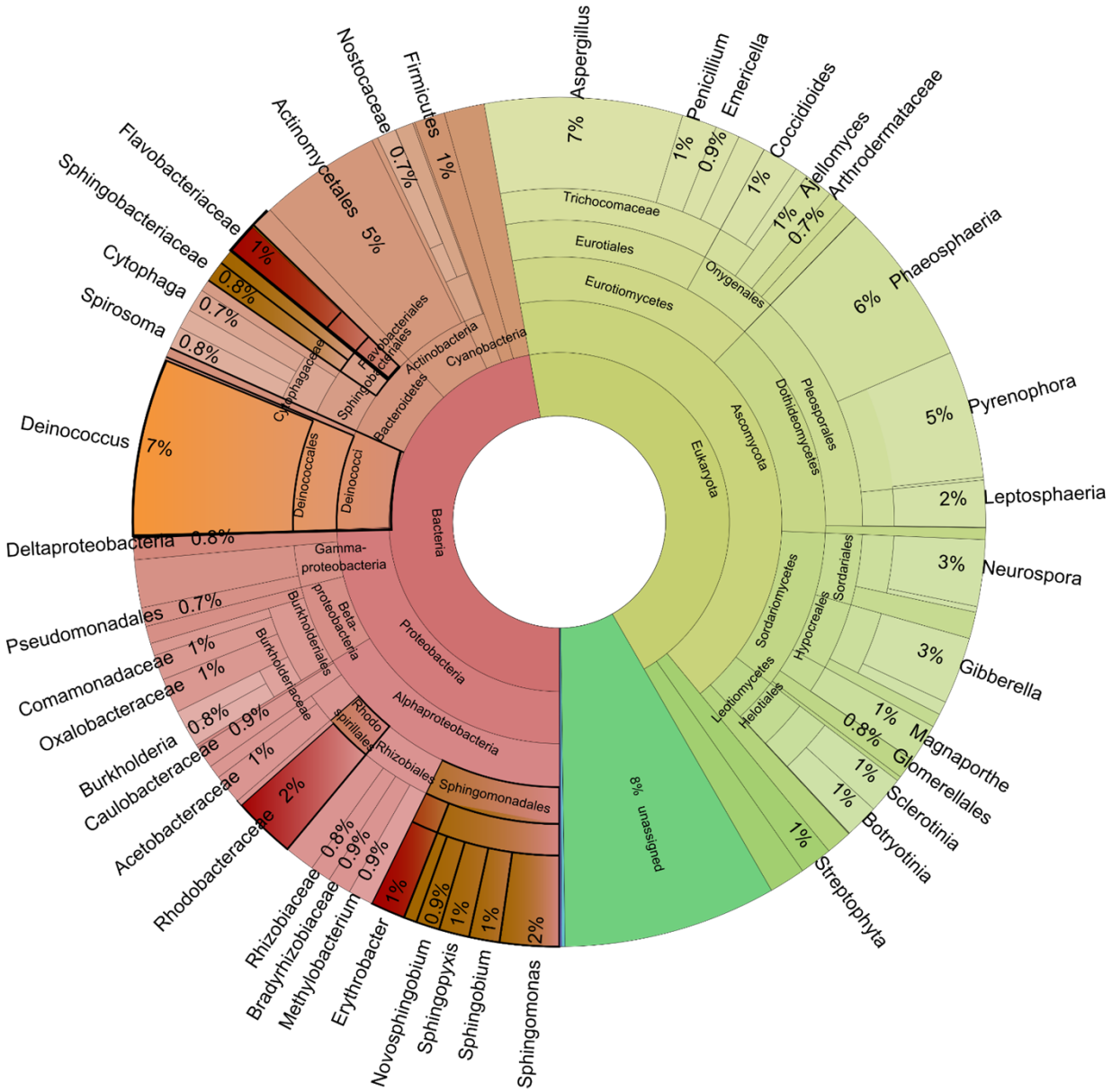


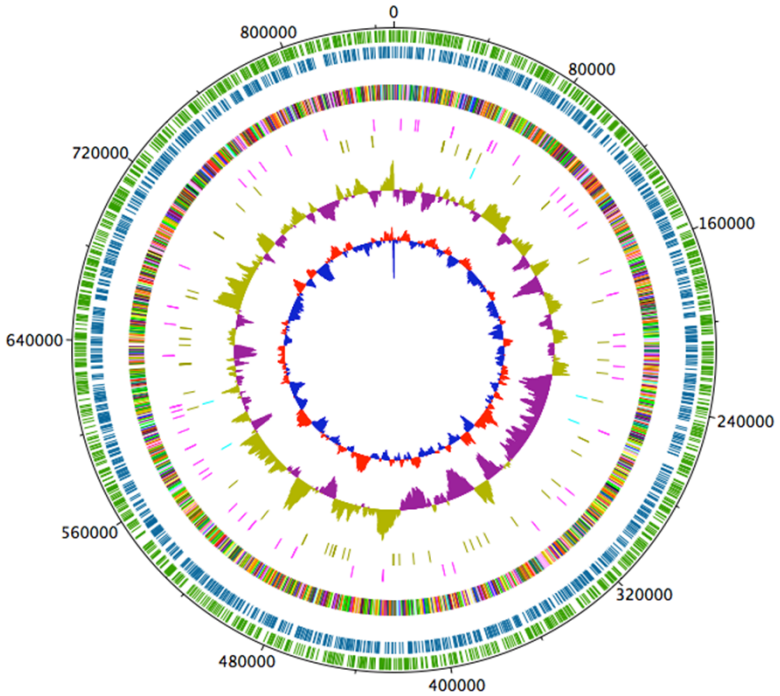


- ✓ Radiation-resistance mechanisms
- ✓ Carotenoids
- ✓ Sphingolipids

- ✓ Phylloplane fungi
- ✓ South-oriented rocks

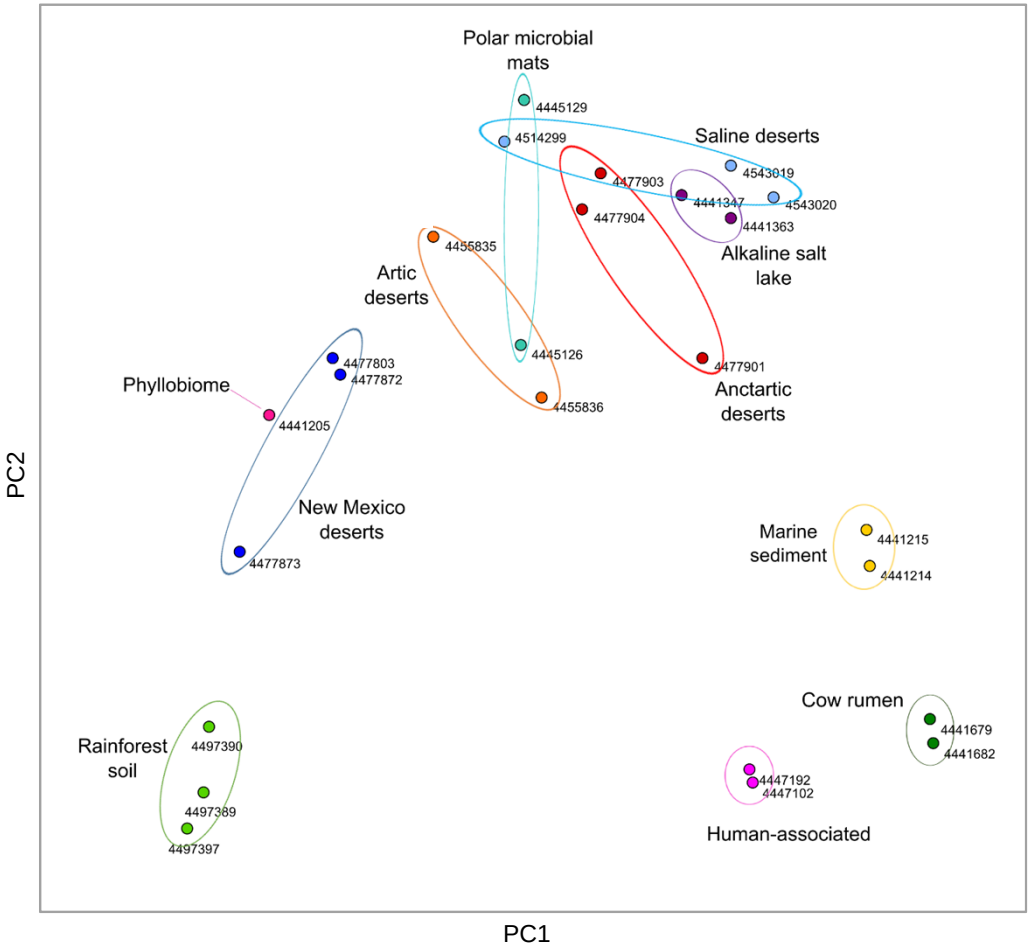
SHOTGUN METAGENOMICS

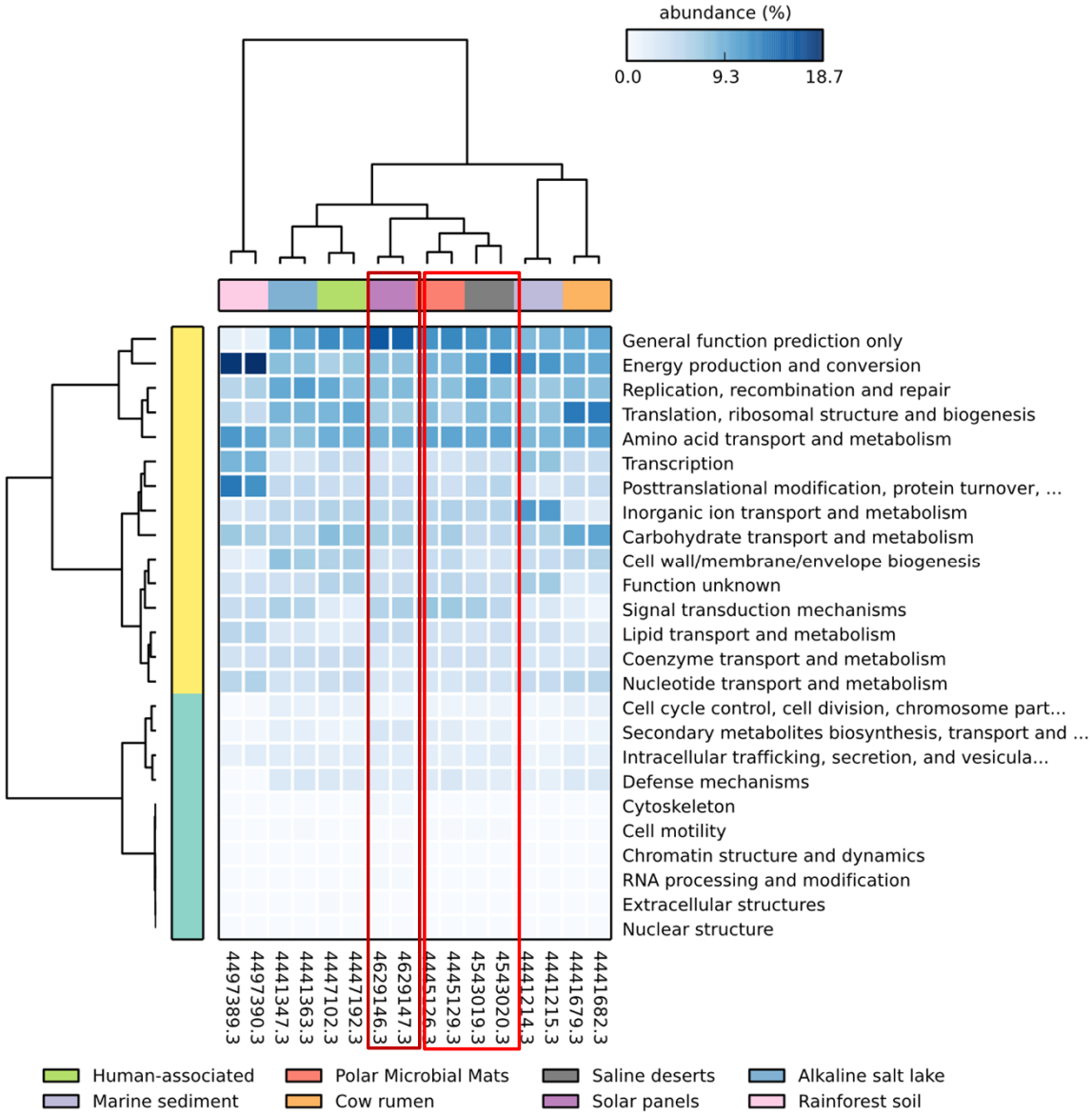




***Deinococcus* pangenome**
(majoritary species not previously sequenced?)

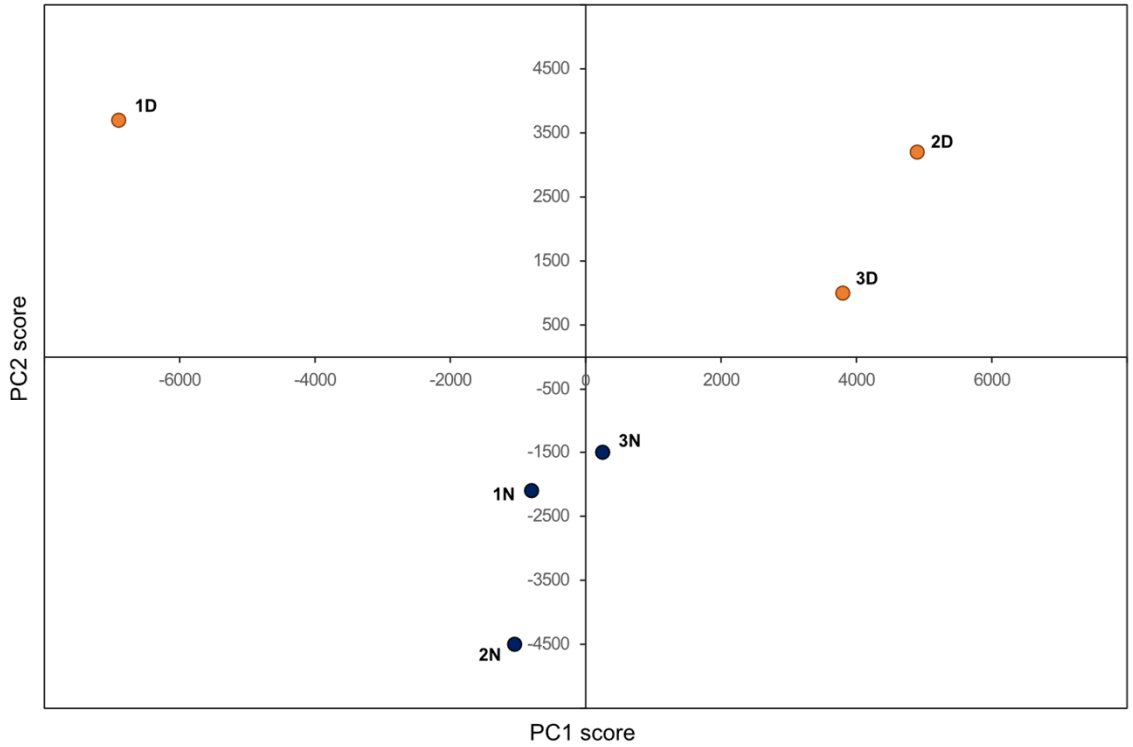
Do solar panels harbor a desert-like microbial community?





METAPROTEOMICS

The microbial communities inhabiting solar panels are biologically active



METAPROTEOMICS

- Response to heat and cold stress
- Maintenance of membrane integrity under harsh conditions
- Growth on surfaces and biofilm formation
- Resistance to drought and salt stress

		Day/Night t-value	p-value	Fold Change Day/Night	Fold Change Night/Day
gi|504554779	cold-shock protein [Geodermatophilaceae]	3,122	0,035	25,986	0,038
gi|656266558	molecular chaperone GroEL [Arsenicicoccus bolidensis]	4,361	0,012	13,665	0,073
gi|503987093	50S ribosomal protein L7/L12 [Niastella koreensis]	3,041	0,038	9,944	0,101
gi|502016435	hypothetical protein [Deinococcus deserti]	3,625	0,022	9,373	0,107
gi|517310793	ATP-1 H+-transporting ATP synthase [Fusarium fujikuroi IMI 58289]	-3,263	0,031	0,473	2,114
gi|656340823	S-layer protein [Deinococcus sp. RL]	-5,991	0,004	0,346	2,890
gi|636360381	major outer membrane lipoprotein 1 [Klebsiella pneumoniae MGH 64]	-2,847	0,047	0,189	5,280
gi|657196340	50S ribosomal protein L7 [Acidiphilium angustum]	-3,031	0,039	0,188	5,326
gi|499563314	F0F1 ATP synthase subunit beta [Synechococcus elongatus]	-2,968	0,041	0,150	6,686
gi|493585871	diguanylate cyclase [Frankia sp. EUN1f]	-2,884	0,045	0,116	8,630
gi|398394263	isocitrate dehydrogenase [NAD] sub 1 [Zymoseptoria tritici IPO323]	-16,097	0,000	0,066	15,147
gi|518290483	molecular chaperone GroEL [Roseomonas]	-9,372	0,001	0,057	17,594
gi|618851123	membrane-bound proton-translocating pyrophosphatase [Clostridium tetanomorphum DSM665]	-3,028	0,039	0,024	40,965

SOLAR PANELS AS URBAN MICRO-DESERTS

The microbial communities present on the solar panels of a Mediterranean city are:

- Highly diverse
- Biologically active
- Adapted to harsh environmental conditions
- Similar to those present in highly irradiated habitats such as deserts and polar environments

The GOLLUM PROJECT

Characterising subterranean bacterial communities in depth(s)

THE GOLLUM PROJECT

The Gollum Project aims at studying the microbial communities inhabiting the very inside of rocks

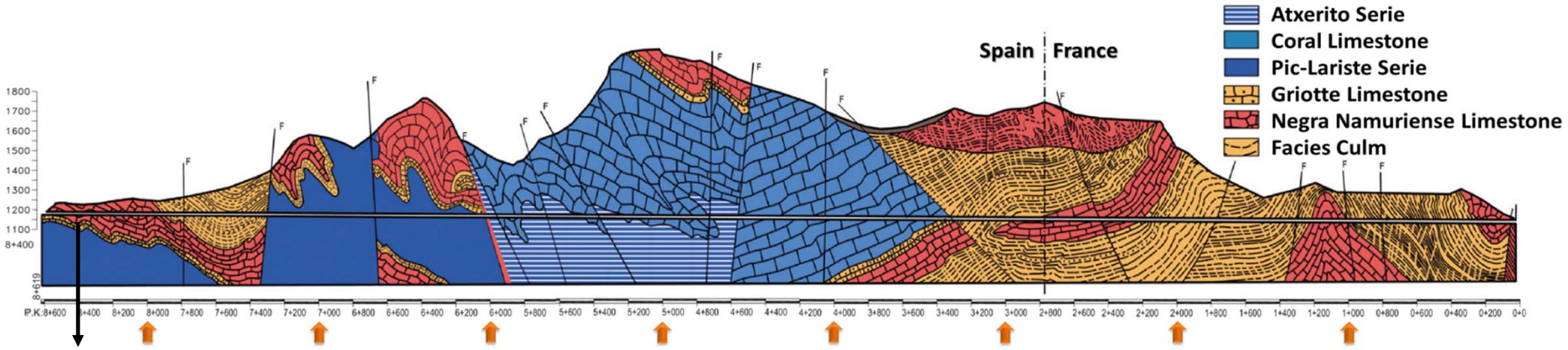
Is such an extreme environment able to host life?

- ✓ Chinese Continental Scientific Drilling Project → Detection of bacteria up to -2000 m
- ✓ Iberian Pyrite Belt Subsurface Life (IPBSL) Project → Metabolically active microbial communities at more than -600 m

THE GOLLUM PROJECT

The Gollum Project aims at studying the microbial communities inhabiting the very inside of rocks

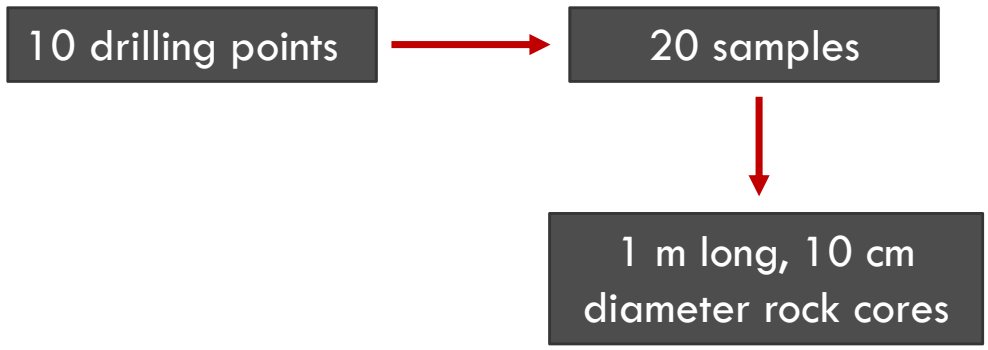
- ✓ Comparison among different rock types and depths



THE GOLLUM PROJECT

The Gollum Project aims at studying the microbial communities inhabiting the very inside of rocks

- ✓ Comparison among different rock types and depths



THE GOLLUM PROJECT

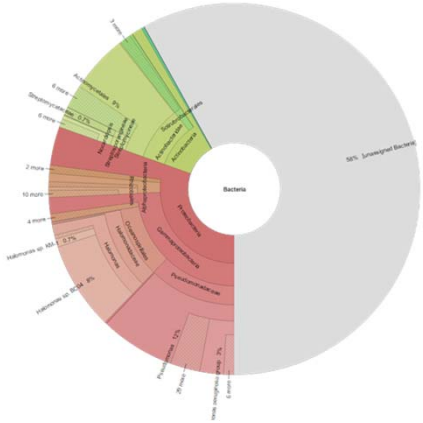
Sampling → DNA isolation → Sequencing → Analysis

Somport tunnel, Canfranc

Rock pulverization & treatment with specialized kits

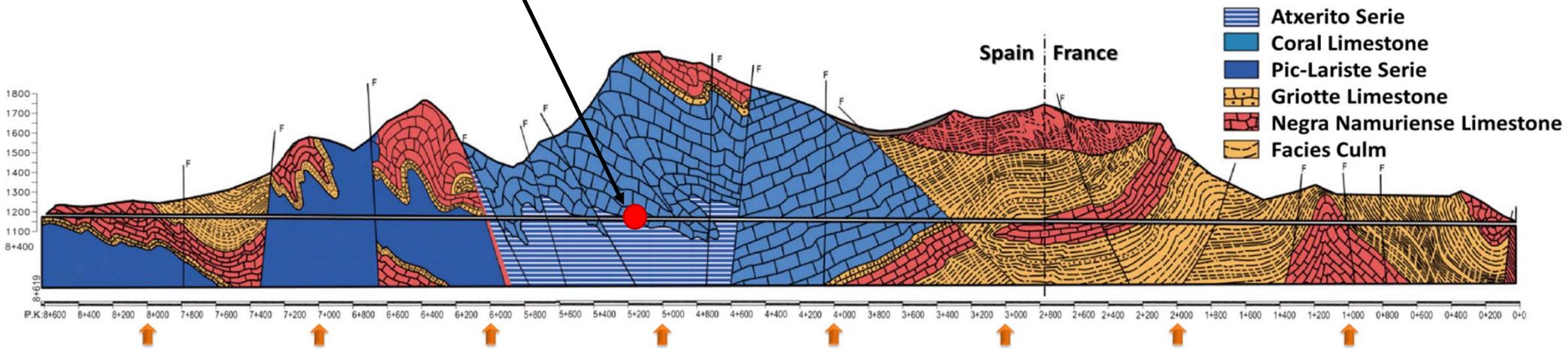
Shotgun Metagenomics

Taxonomic & Functional diversity



THE GOLLUM PROJECT

Sampling *test*

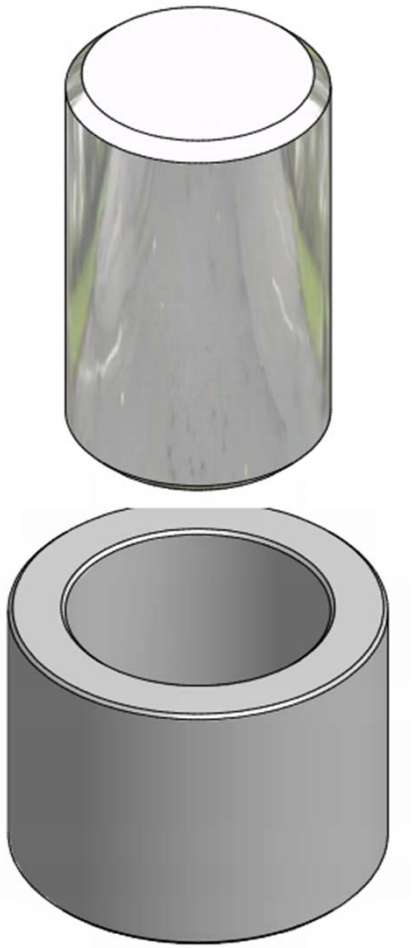


THE GOLLUM PROJECT

Sampling *test*

THE GOLLUM PROJECT

DNA isolation *test*



Sterile steel cylinder and pestle

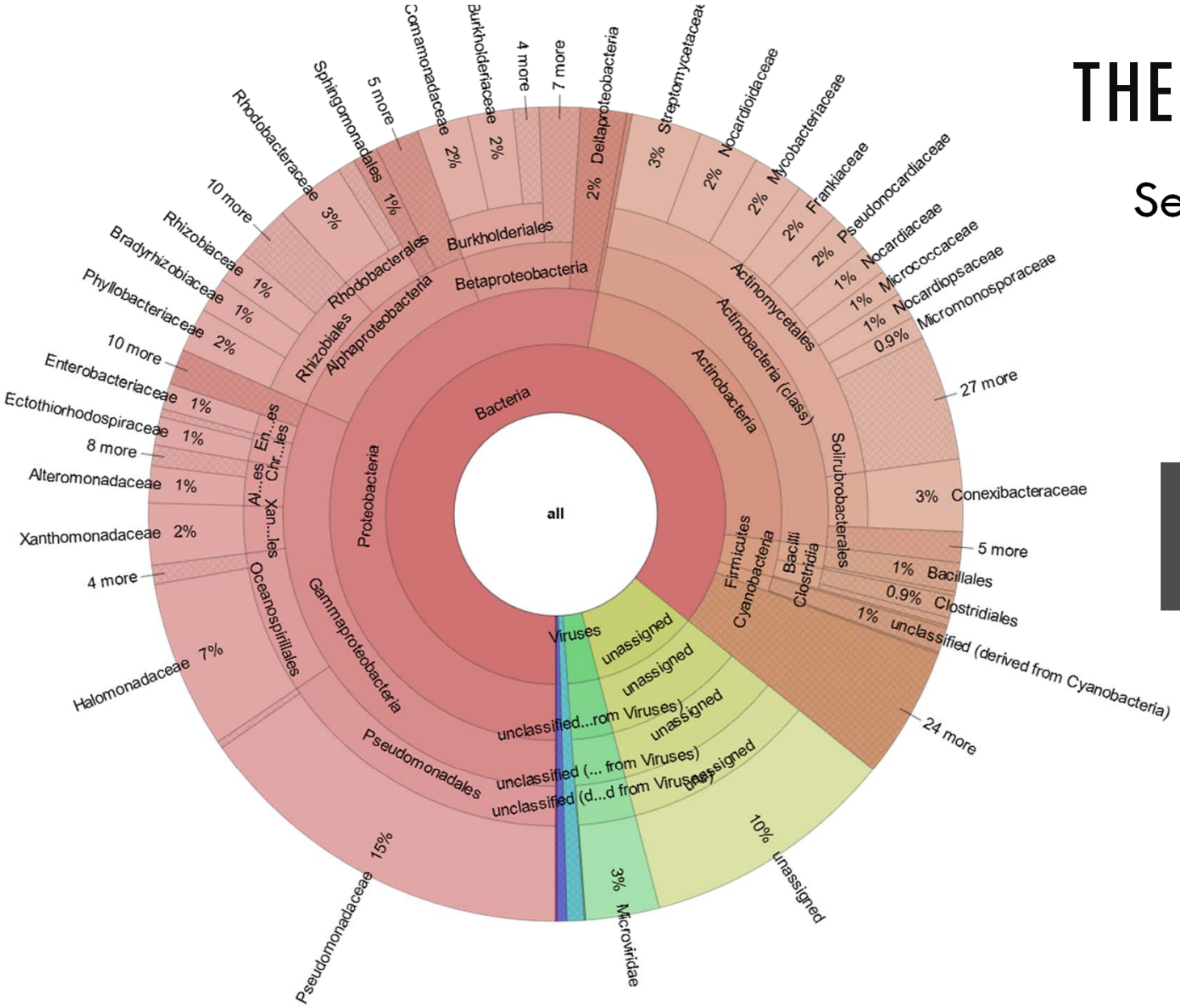
High pressure press (14 Tn per cm^2)

Power Max Soil
(MO BIO laboratories, Inc.)

~ 2 µg of mgDNA

THE GOLLUM PROJECT

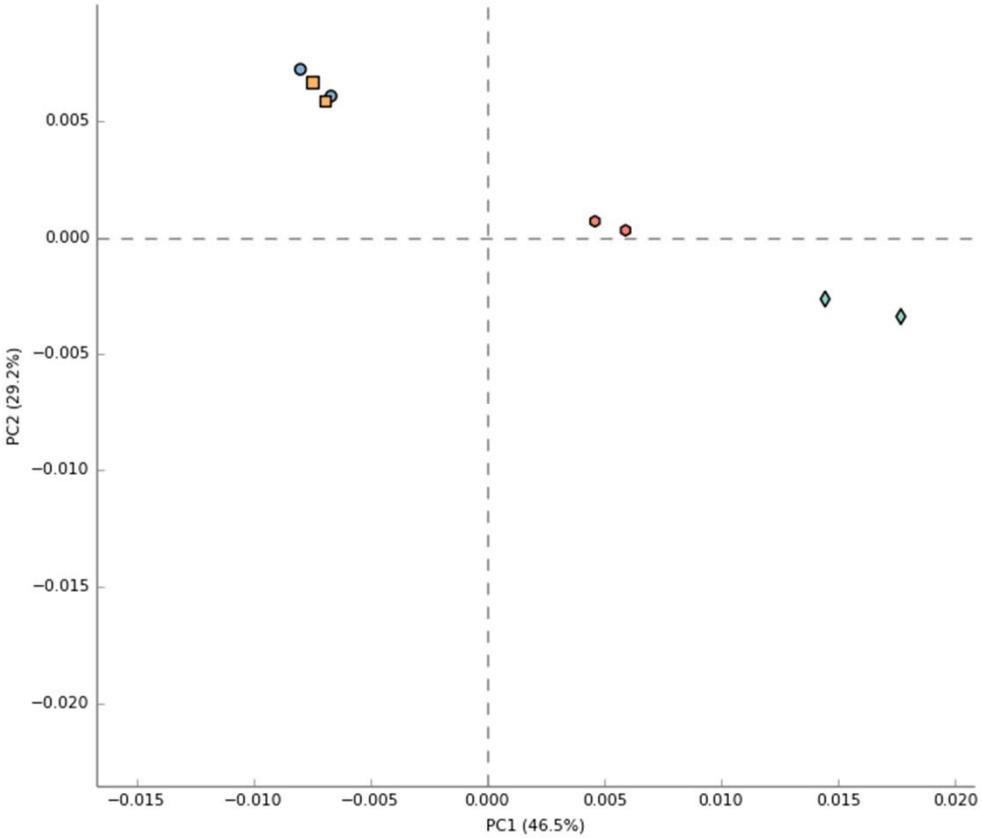
Sequencing & Analysis *test*



Abundance of ***Actinobacteria***, ***Pseudomonas***, and ***Halomonas*** species

THE GOLLUM PROJECT

Sequencing & Analysis *test*



- Meadow
- Saline soil
- Agricultural soil
- Forest soil

THE GOLLUM PROJECT

We want to improve Gollum!

- Establishing collaborations with other underground laboratories to sample more locations
- Sharing samples and data with other groups in order to extend the analysis (i.e.: single-cell genomics)

PEOPLE IN THE GOLLUM PROJECT

Manuel Porcar

Pedro Dorado-Morales

Kristie Tanner

Cristina Vilanova

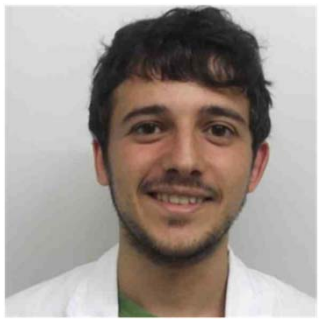
Christian Abendroth

Carlos Peña

César Gracia

Daniel Martínez

José Manuel Martí



Thank you very much
for your attention!