

Alla ricerca di un equilibrio tra Uomo e Terra

Percorsi interdisciplinari per la sostenibilità ambientale,
economica e socio-culturale

Alieni terrestri: gli Archaea ipertermofili dalla ricerca astrobiologica alle biotecnologie industriali

Beatrice Cobucci Ponzano

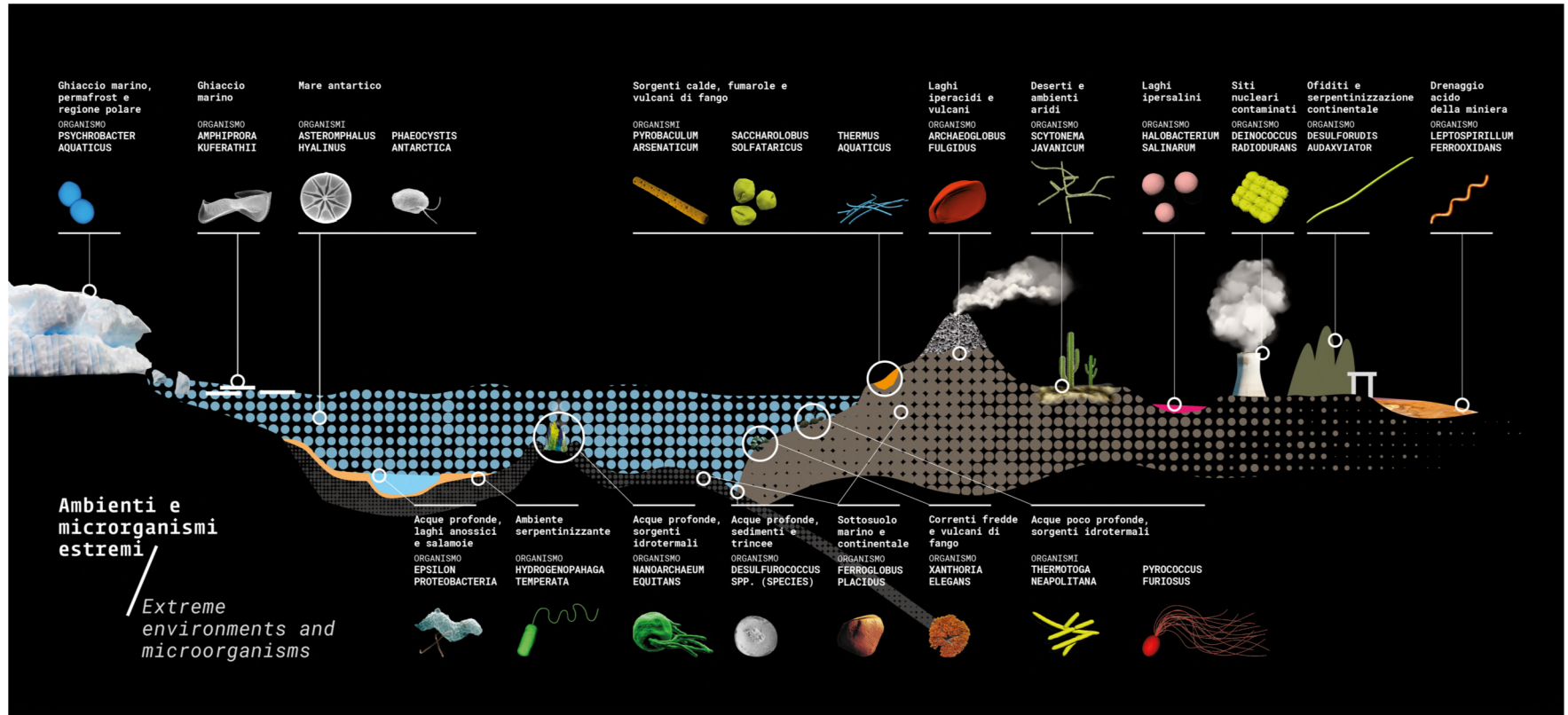
Federica De Lise, Mauro Di Fenza, Luisa Maurelli

Istituto di Bioscienze e BioRisorse - Napoli

Limits of the biologically relevant physical variables:

Life flourished on Earth in an incredibly wide range of environments.

Except for centers of volcanic activity, all the surface of our planet is a biosphere.



Temperature: -40 to + 115°C (stratosphere and hydrothermal vents, respectively)

Pressure: ≤ 120 Mpa (deep sea)

pH: ≈ 1 < pH < 11 (for acidic and alkaline biotopes)

What are extremophiles?

In an anthropocentric vision, *extremophiles* are organisms living at conditions inhospitable for human beings.

Psychrophiles organisms



Mesophilic organisms



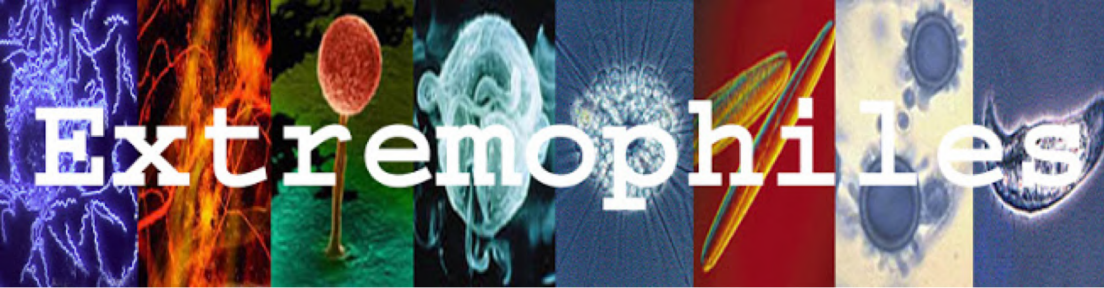
(Hyper)Thermophilic organisms



-15° C

Temperature

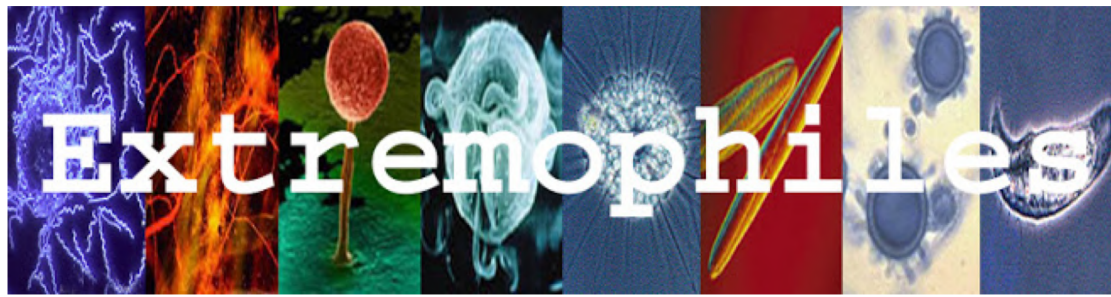
+120° C



Extremophiles

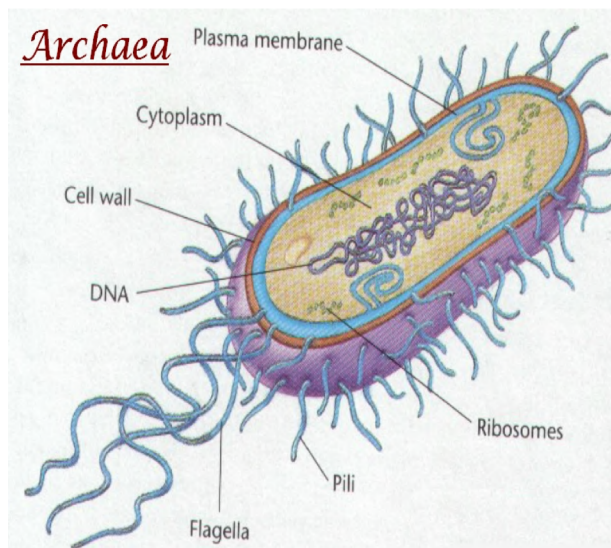
- Definition - Lover of extremes: They **REQUIRE**, not tolerate, this extremes to live
- Temperature extremes
 - boiling or freezing, 100°C to -1°C (212°F to 30°F)
- Chemical extremes
 - vinegar or ammonia ($<5\text{ pH}$ or $>9\text{ pH}$)
 - highly salty, up to ten times sea water





**Most extremophiles identified to date are members of the Archaea,
but not all Archaea are extremophiles**

Archaea are prokaryotes, meaning that they have no cell nucleus or any other membrane-bound organelles in their cells.

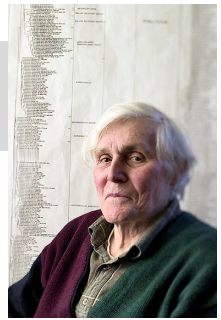


Archaea were initially
classified as bacteria, receiving
the name archaebacteria

"The Woesian Revolution".

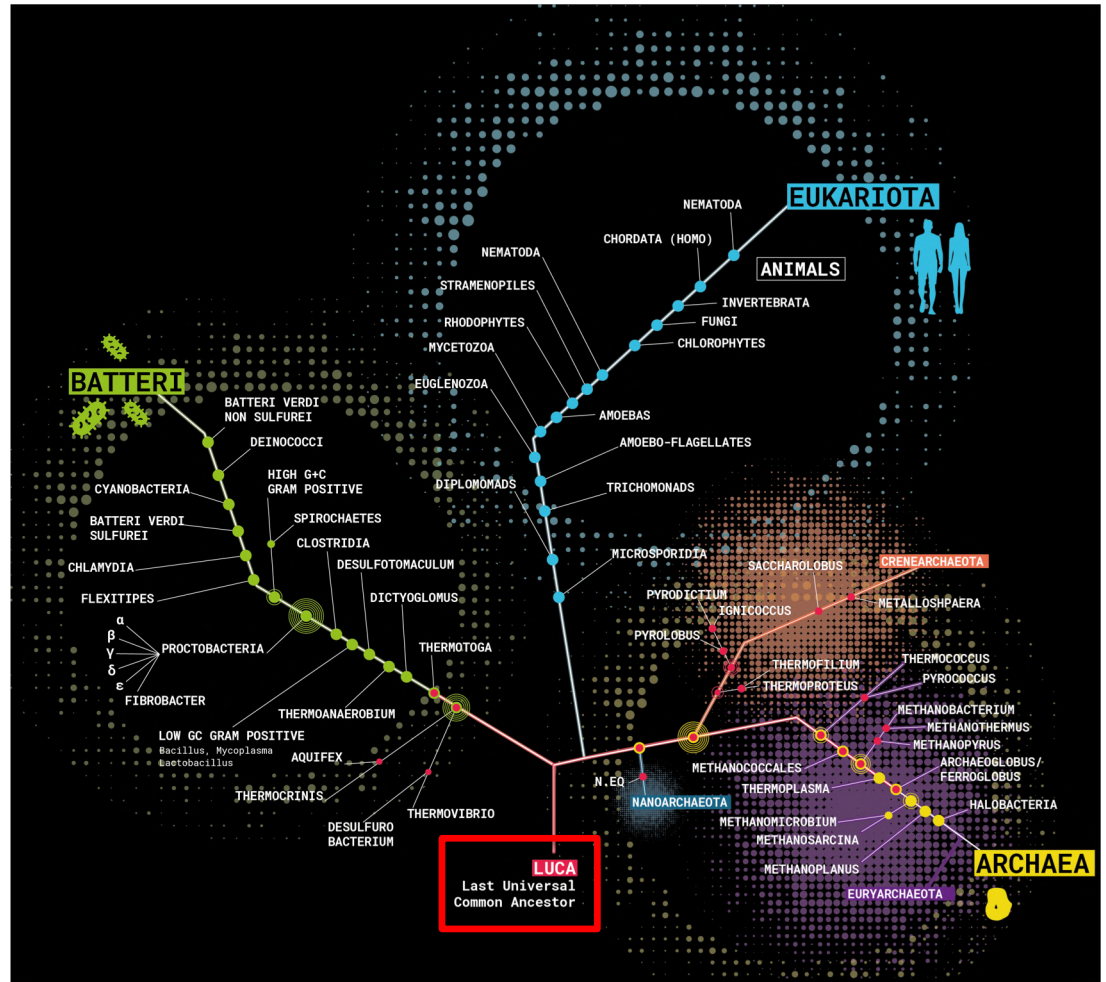
Three Domains of Life

Archaea were first classified as a separate group of prokaryotes in 1977 by Carl Woese and George E. Fox in phylogenetic trees based on the sequences of ribosomal RNA (rRNA) genes.

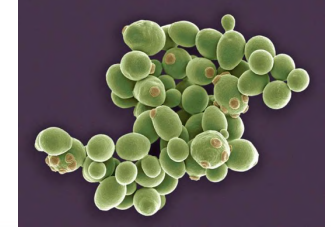
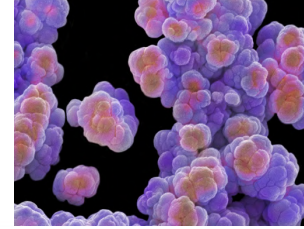
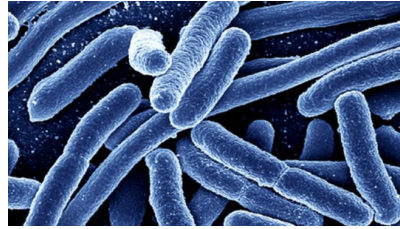


A speculatively rooted tree for rRNA genes, showing the three life domains Bacteria, Archaea, and Eucaryota

and linking the three branches of living organisms to the **LUCA**



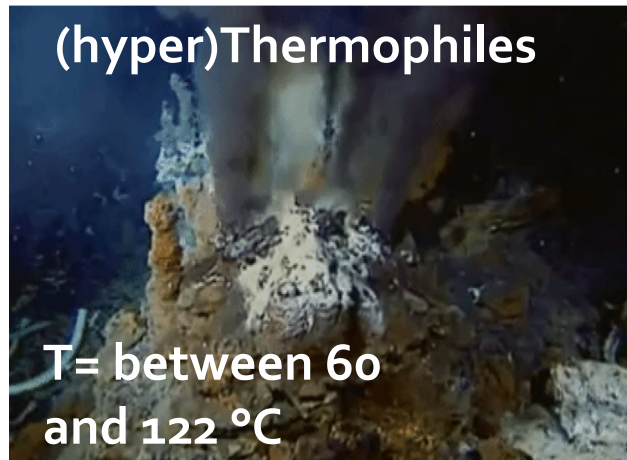
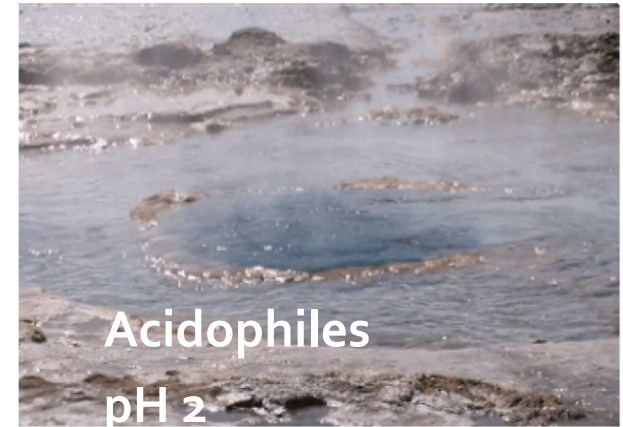
Comparison to other domains



Trait	Bacteria	Archaea	Eukarya
Carbon linkage of lipids	Ester	<i>Ether</i>	Ester
Phosphate backbone of lipids	Glycerol-3-phosphate	<i>Glycerol-1-phosphate</i>	Glycerol-3-phosphate
Metabolism	Bacterial	Bacterial-like	Eukaryotic
Nucleus	No	No	Yes
Organelles	No	No	Yes
Spliceosomal introns	No	No	Yes
Telomeres	No	No	Yes
Chromosome shape	Mostly circular	Circular	Linear
DNA replication	Bacterial	Eukaryotic-like	Eukaryotic
Transcription	Bacterial	Eukaryotic-like	Eukaryotic
Translation	Bacterial	Eukaryotic-like	Eukaryotic

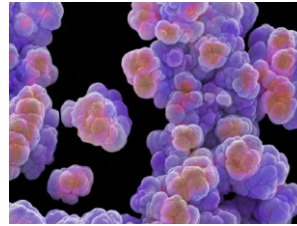
up to 15% of the proteins encoded by any one archaeal genome being unique to the domain, although most of these unique genes have no known function.

Habitat of Archaea

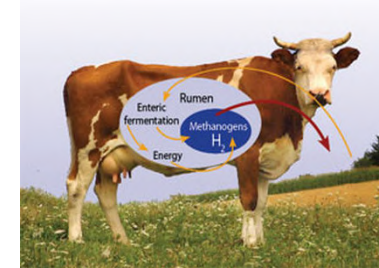


Methanogens

- Some are extremophiles, thriving in hot springs, hydrothermal vents, hot desert soil, and deep subterranean environments.
- Others, are mesophiles, thriving in **human gut**, and **digestive tract of ruminants** where they help digesting polysaccharides, or complex sugars.
- **Cannot even survive in oxygen**
- **Produce methane** as a byproduct of metabolism in absence of oxygen



Methanosarcina



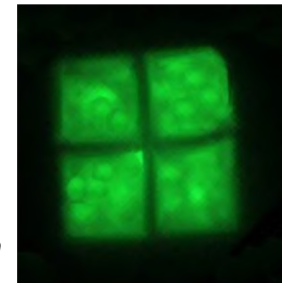
Halophiles - High Salt Environments

- Extreme halophiles **require at least 1.5 M NaCl** for growth
- **Cell lysis occurs below 1.5 M**
- Halophiles achieve the necessary osmotic balance by one of two strategies: (1) accumulating K⁺ in the cytoplasm as a 'salt-in' strategy or (2) excluding salts by synthesizing compatible organic solutes, such as polyols, amino acids, sugars, and betaines.



Halobacterium

Haloquadratum



Alkaliphiles - High Salt Alkaline Environments

- Very few organisms can tolerate alkaline conditions (to date only alkalophilic prokaryotes have been isolated)
- Alkaliphile adaptations: pumps to pump out OH^- , efficient Na^+/H^+ to provide internal H^+ , modified membranes

pH 9.0: Soda Lakes

KENYA'S MASSIVE SODA LAKE

80% covered in soda



Acidophiles

Acidophiles or **acidophilic organisms** are those that thrive under highly acidic conditions (usually at pH 2.0 or below)



pH 0-1 of waters at Iron Mountain

They have evolved extremely efficient mechanisms to pump protons out of the intracellular space in order to keep **the cytoplasm at or near neutral pH**.

Therefore, intracellular proteins do not need to develop acid stability through evolution



Psychrophiles

- Over 75% of Earth's biosphere is permanently cold ($< 5^{\circ}\text{C}$)
- Much of the life present in the cold environs is **planktonic growth of bacteria and archaea** in frigid marine waters

Cold adaptations:

- more fluid membranes
- more structurally flexible proteins



Thermophiles and hyperthermophiles

High temperature

Thermal vents and hot springs

May go hand in hand with chemical extremes



S. solfataricus



- The upper temperature in which microorganisms are reported to be **metabolically active is 122°C** (*Methanopyrus kandleri*)
- Current environmental and theoretical studies suggest that the **upper limit of life might lay near $\sim 150^{\circ}\text{C}$** , due primarily to the instability of macromolecules above this temperature
- Thermostability : Several different molecular mechanisms of nucleic acids and proteins

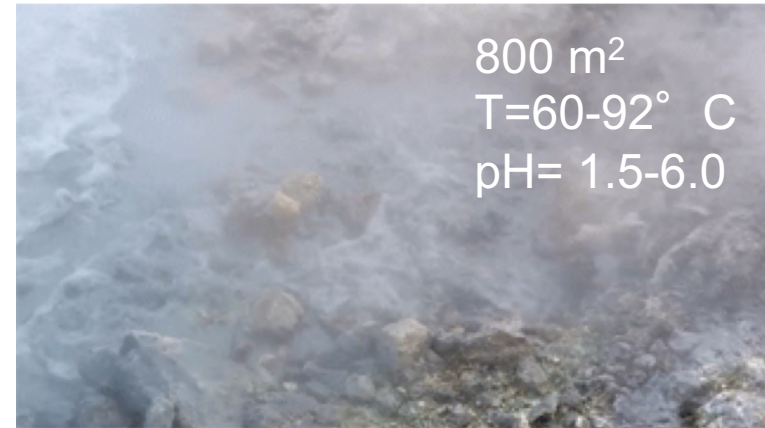
Campi Flegrei (Naples)

The **Campi Flegrei Regional Park** protects an active volcanic area in the Campania region, which is constantly evolving.

One of the main attractions of the **Campi Flegrei** is the **solfatara**, characterized by fumaroles, sources of gas and mineral water, jets of hot mud and earthquakes. The Parco dei Campi Flegrei is aimed at the enhancement and protection of biodiversity and the creation of a sustainable development model, based on the enhancement of the Archaeological, Landscape, Naturalistic, Historical and Thermal Heritage.



Solfatara Pisciarelli Agnano (Naples)



Solfatara Pisciarelli Agnano (Naples)



Study of extremophiles in astrobiology

- Evolutionary history of life on Earth

Origin, evolution and distribution of life.

Nature of the Last Universal Common Ancestor (LUCA)

- Models of pioneering life-form on other planets

Astrobiology roadmap 2015, NASA

**Astrobiology research has continued
to expand the limits of life**



Habitability has typically been defined as the potential for an environment to support life

The main aim of habitability investigations is to understand the limits of life on Earth

This is critical for where and how we should search for life elsewhere!

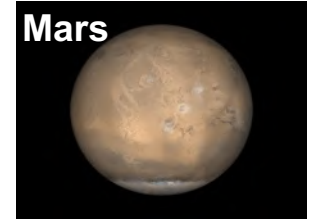
In the past few decades our definition of habitability has expanded with the discovery of life in extreme environments



Exoplanets

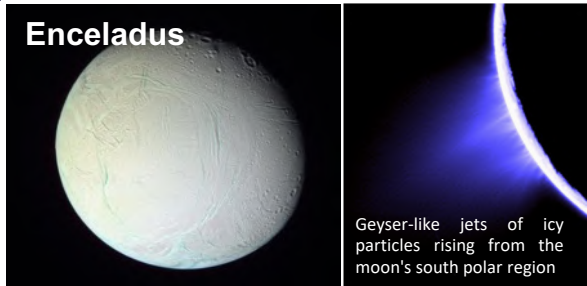
Extremophiles	Conditions	Earth Habitat	Relevant environment(s)
Psychrophiles	Low temperature	Snow, ice, sediment	Ice shells of Europa and Enceladus ; poles of Mars
Halophiles	High salinity	Sea ice inclusions, saline lakes, evaporation ponds	Subsurface oceans of Europa , Titan , and Enceladus
HyperThermophiles and Piezophiles	High pressure	Hydrothermal vents of the ocean floor	Ocean floors of Europa (hydrothermal?)
Xerophiles	Low water activity	Atacama desert, rock surface	Surface of Mars
Radiation-tolerant microorganisms	High radiation	Nuclear reactor water, cores	Surface of Europa
Chemolithotrophs	Liquid hydrocarbon matrix	Pitch Lake, oil seeps	Hydrocarbon lakes of Titan

Mars



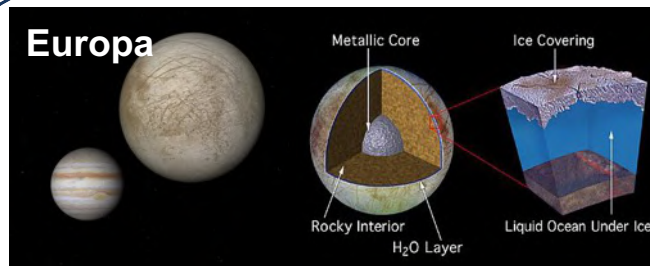
- Surface temperatures: about -143°C up to $+35^{\circ}\text{C}$.
- Low atmospheric pressure.
- Low thermal inertia of Martian soil.
- Atmosphere composition: 95% CO_2 , 3% N_2 , 1.6% Ar, traces of O_2 and H_2O .

Enceladus



- Atmosphere composition: 91% H_2O vapour, 4% N_2 , 3.2% CO_2 , 1.7% CH_4 .
- Geologically active today (geyser-like jets).
- Analysis of the outgassing suggests that it originates from a body of sub-surface liquid water.

Europa



- Tenuous atmosphere composed mostly of molecular oxygen (O_2).
- The surface pressure of atmosphere is $0.1\ \mu\text{Pa}$, (10^{-12} times that of the Earth).
- Life could exist in its under-ice ocean, in an environment similar to Earth's deep-ocean hydrothermal vents.

Titan

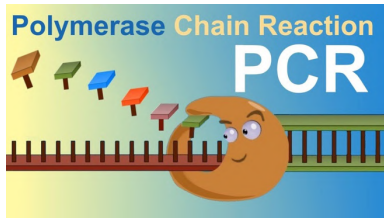


- Surface temperature: about -179.2°C
- Atmospheric composition: 98.4% N_2 , 1.4% CH_4 and 0.2% H_2
- Little geologic activity

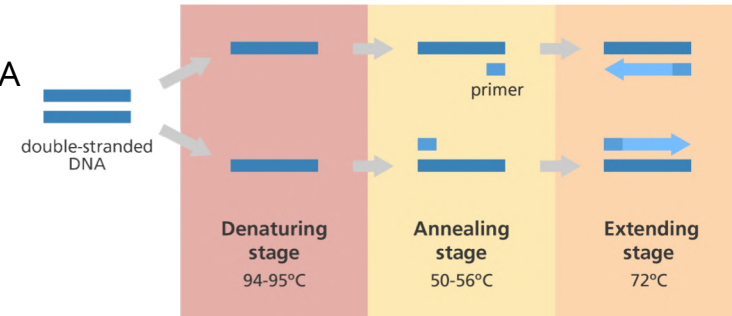
Industrial application of extremophiles

Biocatalysts isolated by these organisms: extremozymes, very resistant to extreme conditions

Extremophiles impact in molecular biology: Polymerase Chain Reaction (PCR)



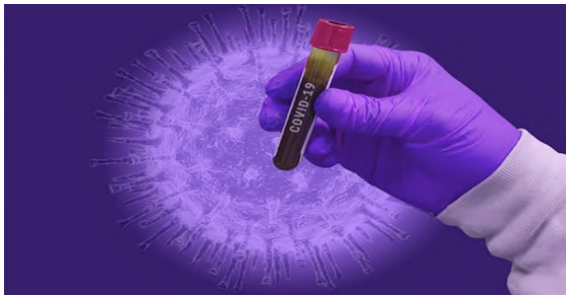
PCR
Requires high temperature to denature DNA
↓
Thermostable DNA polymerase



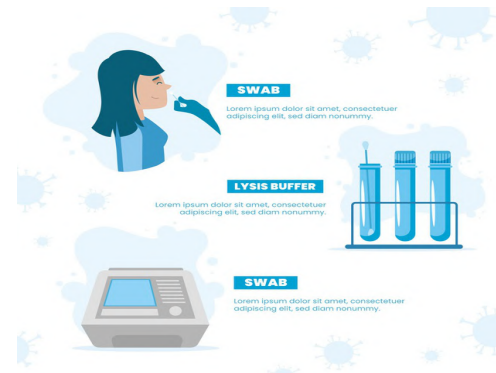
Taq DNA polymerase:
from the bacteria *Thermus aquaticus*



Pfu polymerase:
from the hyperthermophilic archaeon *Pyrococcus furiosus*.



PCR is important for diagnosis:
COVID-19 molecular tests.



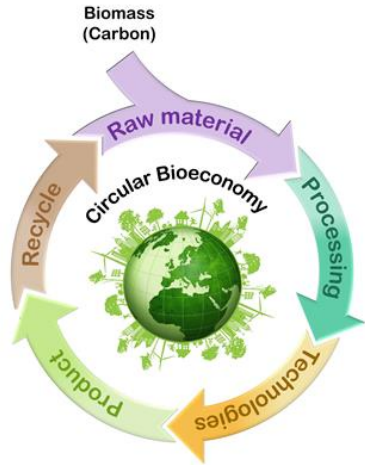
Industrial application of extremophiles

Biocatalysts isolated by these organisms: extremozymes, very resistant to extreme conditions

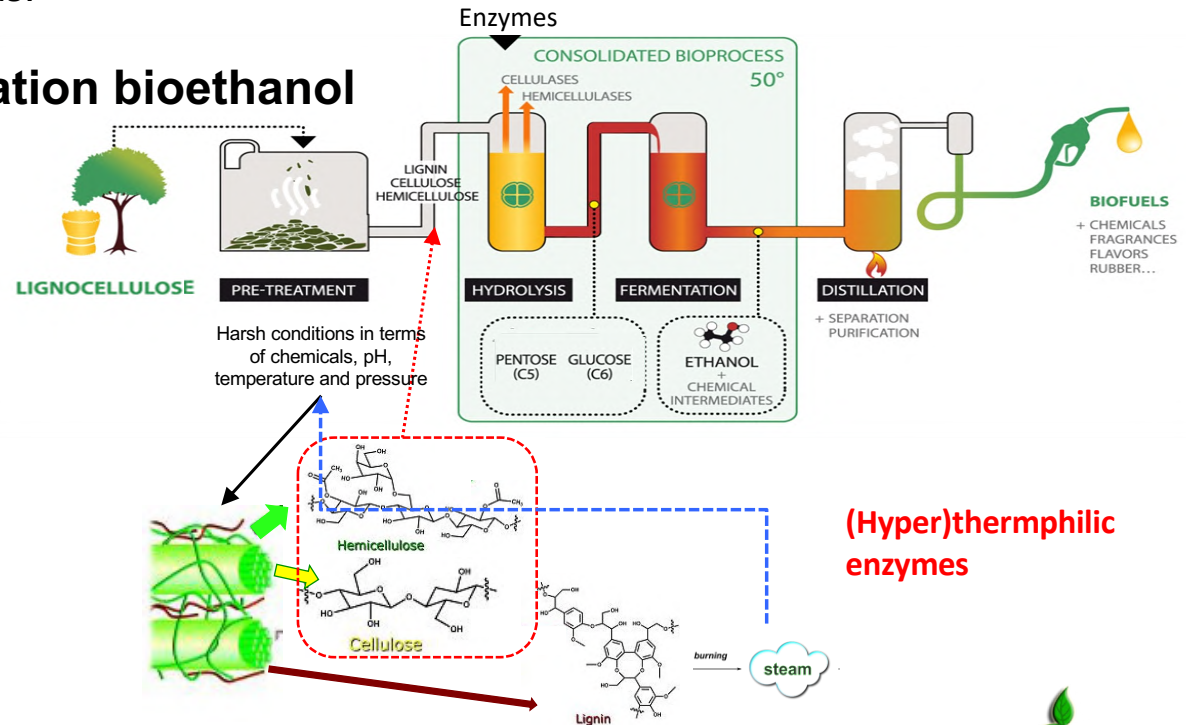
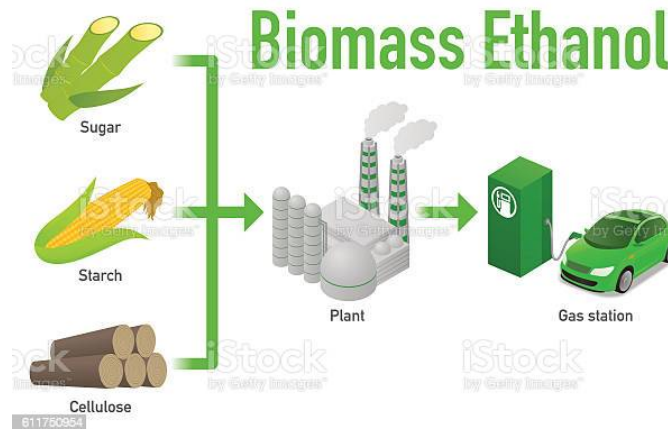
Extremophiles impact in circular bioeconomy

To offer a sustainable, renewable, and commercially feasible alternative to fossil fuel-based products by using plant biomass residues, reducing greenhouse emissions.

This approach relies on the conversion of plant polymers into commercially relevant products such as biofuels, chemicals, food ingredients and pharmaceuticals.



Production of second generation bioethanol



Industrial biotechnology

- is based on the application of biological systems (i.e. enzymes and microorganisms) for industrial purposes
- is based on the creation of synergy between chemical engineering and biology
- Sustainable technology

EUROPE

1. High level of science, innovation, and talent
2. Hotspots in a fragmented landscape



Where we are



Where we want to be



www.ibisba.eu

www.ibisba.eu | info@il

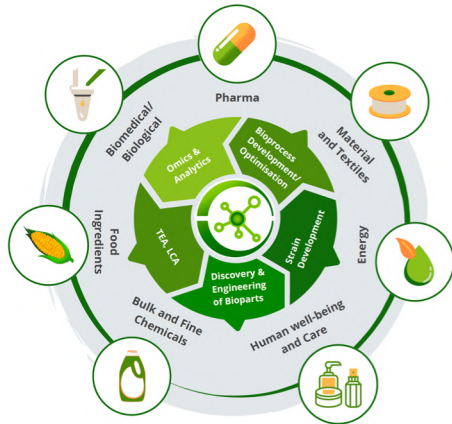


Figure 1. IBISBA services are relevant to a wide array of market sectors covered by biotechnology.

Figure 2 - IBISBA's founding partners are present in eight member states and the UK.



Extremophilic Archaea: take home message

Life flourished on Earth in an incredibly wide range of environments

Most extremophiles identified to date are members of the Archaea,

Lover of extremes: They REQUIRE, not tolerate, this extremes to live

Archaea are closely related to LUCA.....and to Eukarya

The study of the microbial communities populating extreme environments is an interesting approach in astrobiology that may help us to understand the limits of life

Biocatalysts isolated by these organisms: extremozymes, very resistant to extreme conditions

New enzymes for biotechnological applications in agricultural, chemical, biomedical, and biotechnological processes





UNIVERSITÀ DEGLI STUDI
DI NAPOLI FEDERICO II

Extreme Group



National Research
Council of Italy



Andrea
Strazzulli

Beatrice
Cobucci Ponzano

Roberta
Iacono

Federica
De Lise

Nicola
Curci

Marco
Moracci



Luisa Maurelli
Mauro Di Fenza

Funding



Agenzia
Spaziale
Italiana



2019-2023 - PREP-IBISBA Grant agreement ID: 871118 - H2020

2019-2022 - OPPS LIFE IN SPACE - Italian Space Agency

2017- 2021 IBISBA 1.0 Grant agreement ID: 730976 INFRAIA-02-2017 - H2020

Master Degree

Biology of Extreme Environments



BIOLOGICAL RESOURCES



ASTROBIOLOGY

BIOLOGICAL RESOURCES

ASTROBIOLOGY

www.dipartimentodibiologia.unina.it

www.bioextreme.it