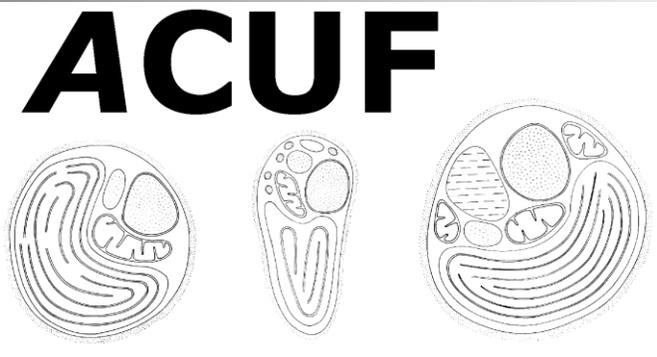




European Culture
Collections' Organisation

Diversity of microalgae in low pH environments: fifty years of the ACUF Collection, from strain to microbiome conservation and exploitation



Antonino Pollio
Bari, 18-20 september 2024
ECCO XLII Meeting
"Microbe & Microbiome Management for a Better Planet"

The algae collection at the University of Naples “Federico II”: from the beginnings to ACUF (1974-2024)

1963. International Phycological Symposium, Zoological Station- Naples

Key people- Kurth Beth and Aldo Merola

Research project: to study the flora and microflora of thermo-acidophilic environments ($\text{pH} < 3.0$) surrounding Naples. The single-celled alga *Cyanidium caldarium* became the focus of these studies.



Professor Aldo Merola (1963)

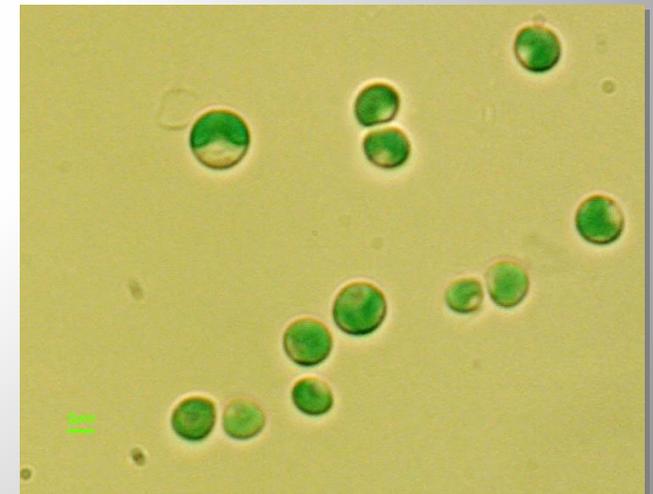
Cyanidium caldarium

In 1933, Lothar Geitler (University of Wien) described *Cyanidium caldarium*, a single-celled alga initially attributed to the Cyanophyceae, today Cyanobacteria.

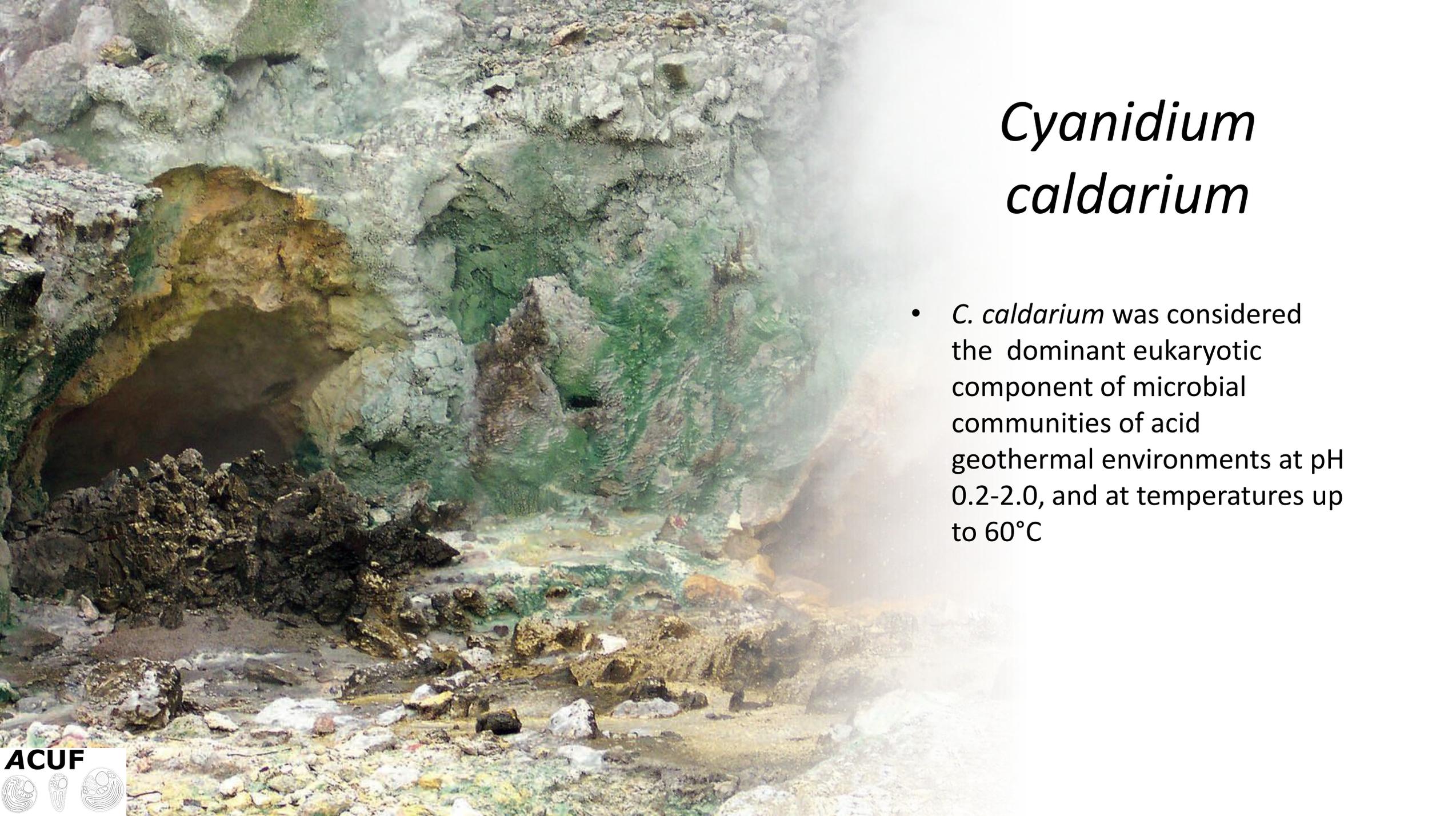


L. Geitler 1899-1990

The taxonomic position of this alga has changed over time, until it found its definitive place among the red algae (Rhodophyta)



Cyanidium caldarium



Cyanidium caldarium

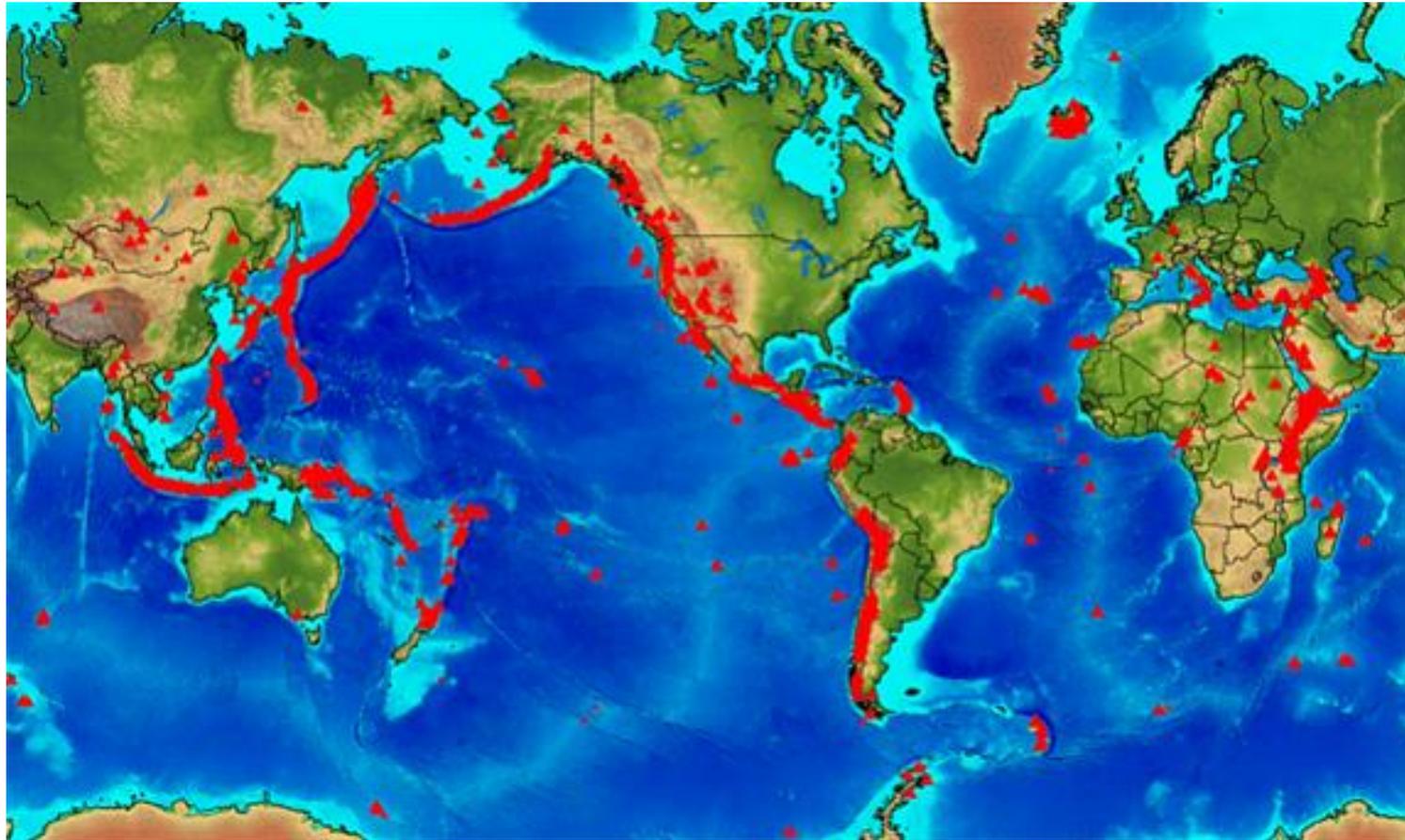
- *C. caldarium* was considered the dominant eukaryotic component of microbial communities of acid geothermal environments at pH 0.2-2.0, and at temperatures up to 60°C



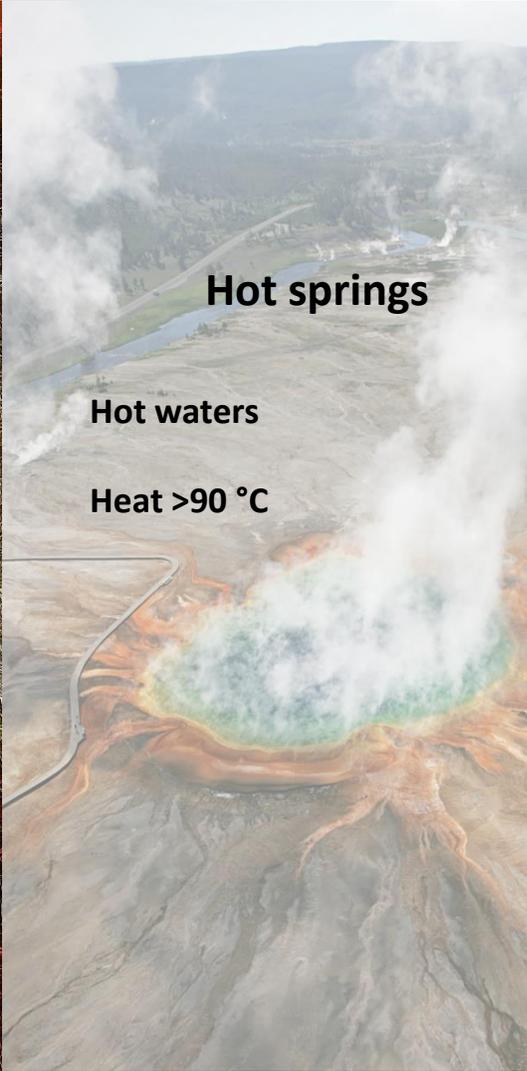
Acid geothermal environments

Acidic geothermal environments resulting from sulfur mineral oxidation are widespread on Earth

(red lines and dots in the figure)

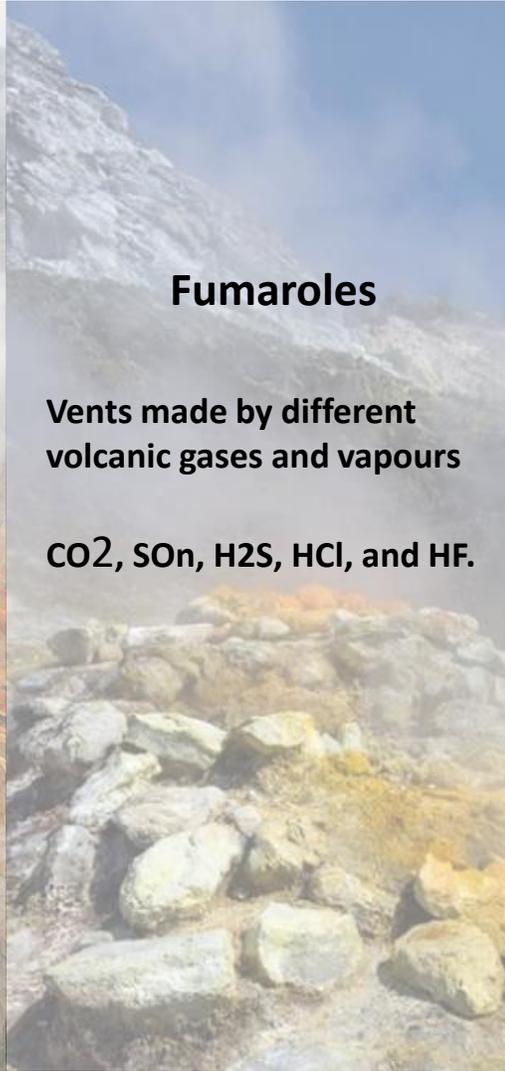


Low pH environments in Italy. Geothermal sites



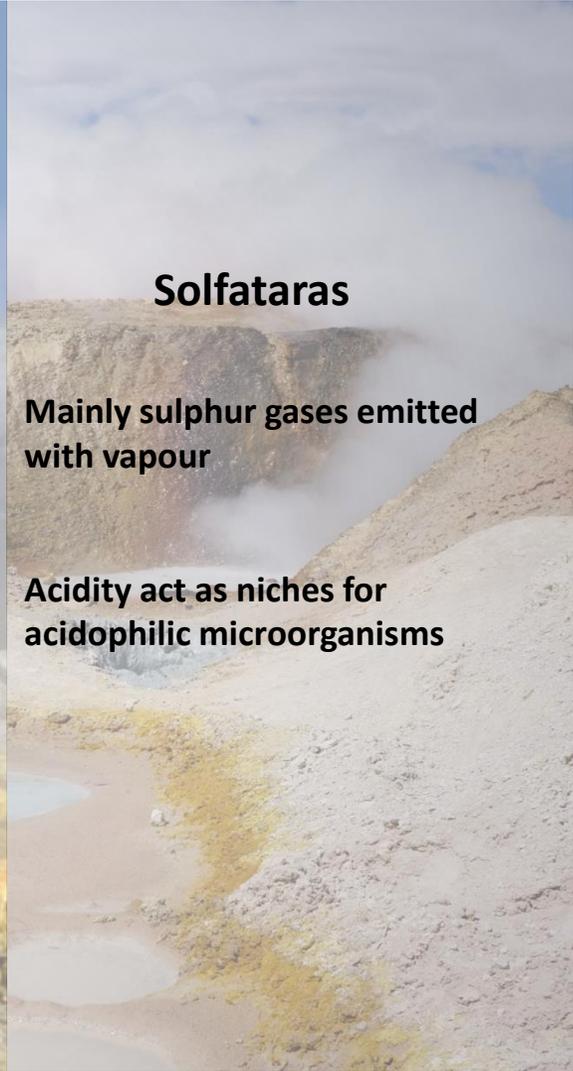
Hot springs

Hot waters
Heat >90 °C



Fumaroles

Vents made by different volcanic gases and vapours
CO₂, SO₂, H₂S, HCl, and HF.



Solfataras

Mainly sulphur gases emitted with vapour
Acidity act as niches for acidophilic microorganisms

Low pH Environments in Italy: sulphur mines

- sulphur deposits are located mainly in Sicily and the Marche and Romagna basins. The geological reason for the abundance of sulphur is the presence of the gypsum-sulphur formation, due to a long phase of evaporation in the Mediterranean Sea that occurred about 6 million years ago

Gill Ovens
Cassibile sulphur mines, Sicily
ACUF expedition, 1975



<https://www.valcesano.com/blog/storia-e-tradizioni/minieradizolfo-visitadicabernardi>

1970's



The culture collection of algae at the Federico II University of Naples and was initially planned as a collection of *Cyanidium caldarium* Geitler (*sensu lato*) strains from different low pH environments of Italy.

A systematic survey of more than 60 sites was undertaken between 1972 and 1978, and about 250 “*Cyanidium*” strains were isolated, becoming the ACUF core.

1973-4 -The first ACUF staff



Exploring low pH environments of Italy (1972-1977)

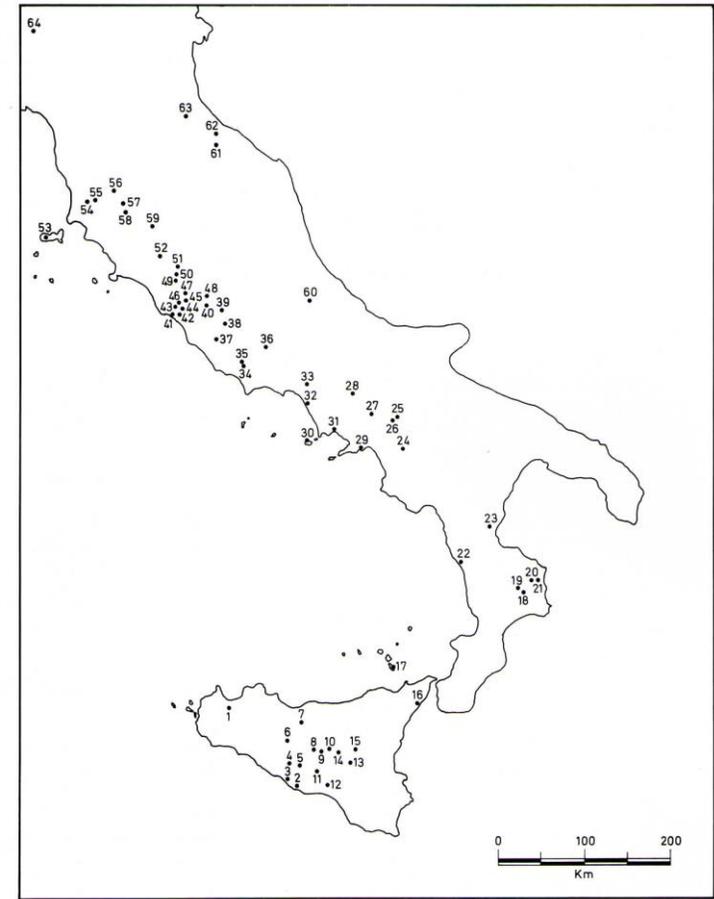


Fig. 1 - Geographic map of peninsular Italy ad Sicily. The numbers indicate the sites of prevalence of the strains





Sampling strategies



Sampling along a pH and temperature gradient

In each hot spring pH, temperature, salinity, heavy metal concentration are highly variable, depending on a number of different factors. This influences the diversity of microbes found in each location, from extreme tolerant to extremophile



epilithic



cryptoendolithic

Sampling at different depth

Two main achievements

- 1. *Cyanidium caldarium* lived together with two other species new to science that were described by R. Taddei and coworkers: *Galdieria sulphuraria* and *Cyanidioschyzon merolae*
- 2. in low pH environments, where temperature is lower than 30 °C, a large array of microorganisms thrives, also at very low pH values (0.6-1.5)

1979-1982 sampling in other Continents

In the late 1970s, the collection began to collect Cyanidiales from other Continents. An ACUF staff collected algal samples in Central America. From these samples were isolated strains that have been maintained in the collection ever since.



Playon de Auachapan (El Salvador)
Los Azufres e Cerro Prieto (Mexico).

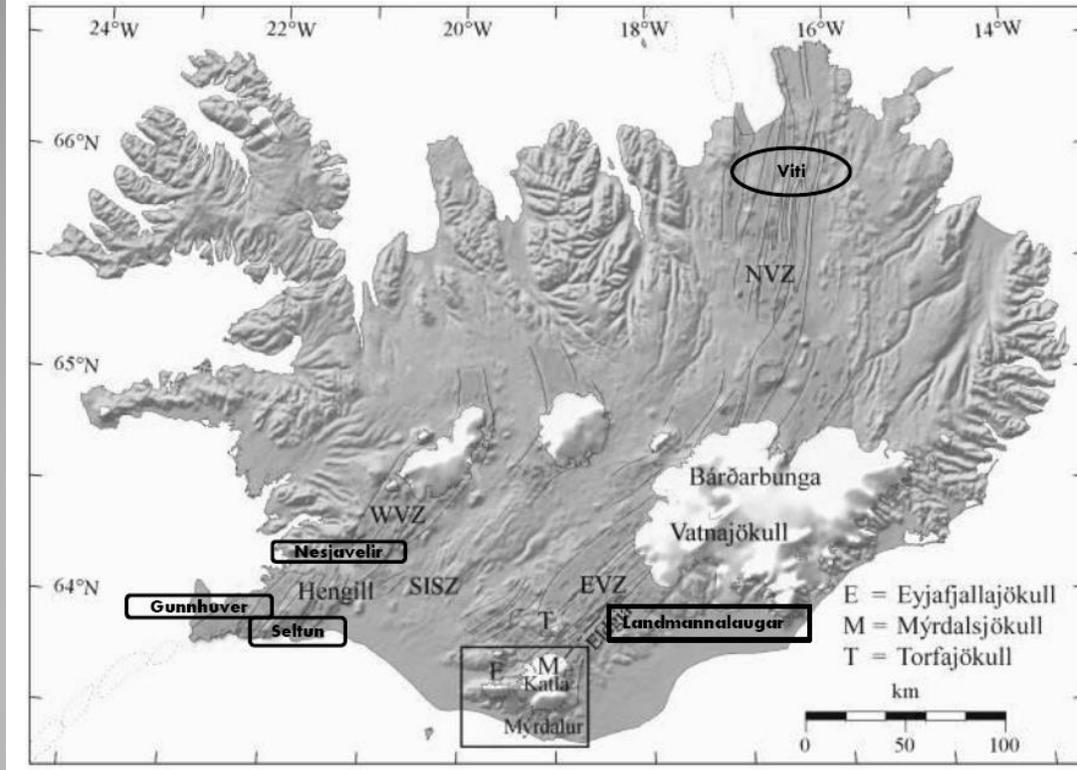
De Luca et al. (1977, 1979)
Gambardella et al. (1980)

1983

The expedition of Professor Paolo De Luca to Mount Lawu, Java (Indonesia) led to the collection of new Cyanidiales strains



Iceland 2005 – Ciniglia Expedition



ST 1 – Gunnhver

ST2 – Seltun

ST3 – Heragerdi

ST4 – Niasjvellir

ST5 – Geyser

ST6 – Viti

ST7 – Bijarnaflag

ST8 – Landmannalaugar





Turkey 2011 Ciniglia, Iovinella, Eren expedition

- Diyarbakir-Cermik (SET-CE)
- Siirt-Biloris (SET-BI9)
- Guclukonak-Hesta (SET-GU)
- Vulcano Nemrut (CET-NE)
- Vulcano Tendurek (CET-TE)
- Agri-Diyadin (NET-DI)
- Manisa-Kula (SWT.KU)
- Manisa-Salihili e Kursunlu (SWT.SL)
- Manisa-Sart (SWT-SR)/Aydin-Germencik (SWT-GE)

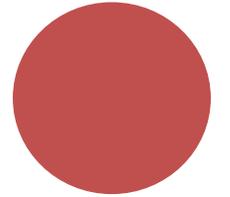


- **2021-present**

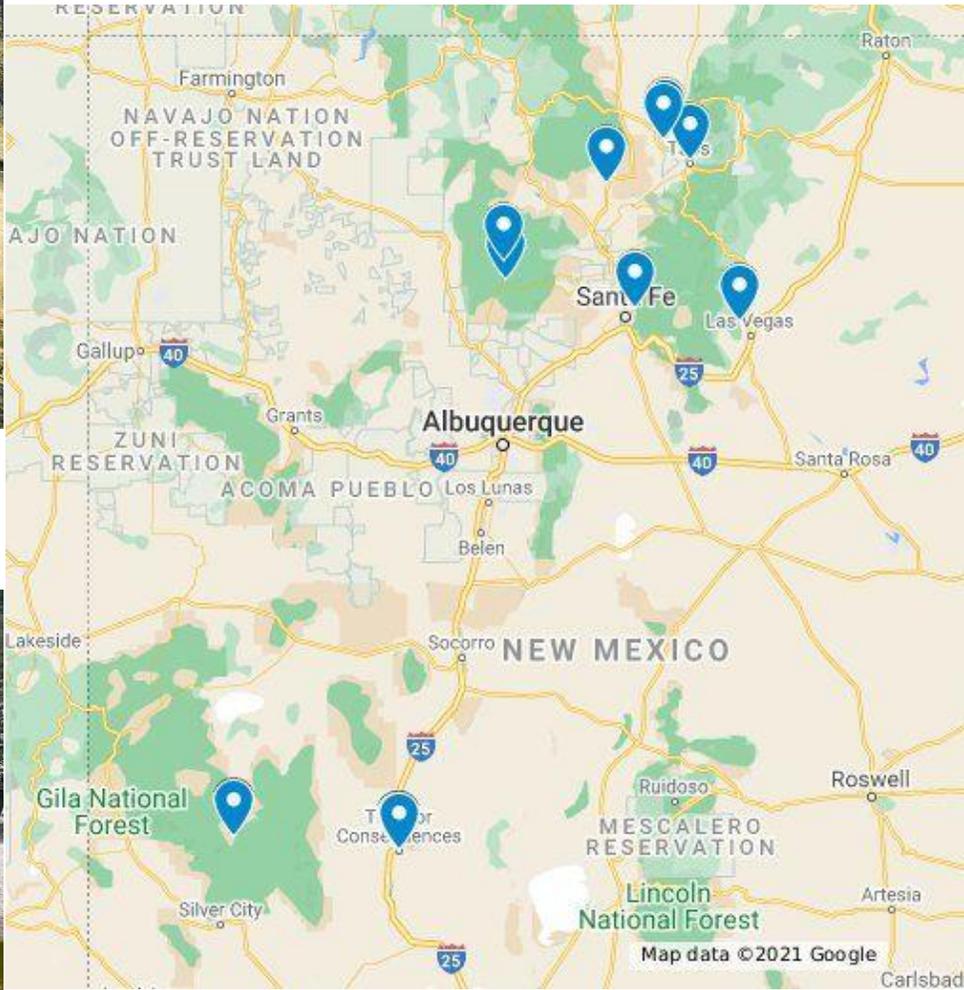
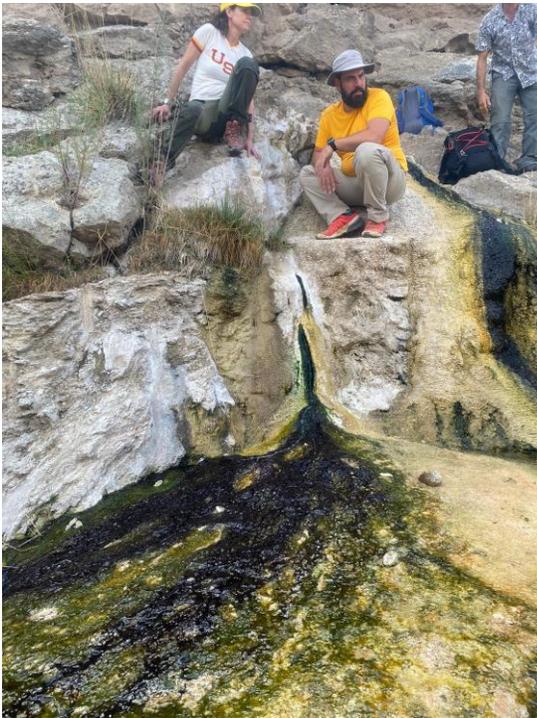
- ACUF joins the **Coevolve** project (leading scientist prof. Donato Giovannelli)

- **Coevolve**, funded by EU ERC Starting Grant, studies the co-evolution of biogeochemically-relevant proteins and trace metal availability in extreme environments

- New hot springs and volcanoes from different Countries (Argentina, Chile, Iceland) have been visited and sampled.

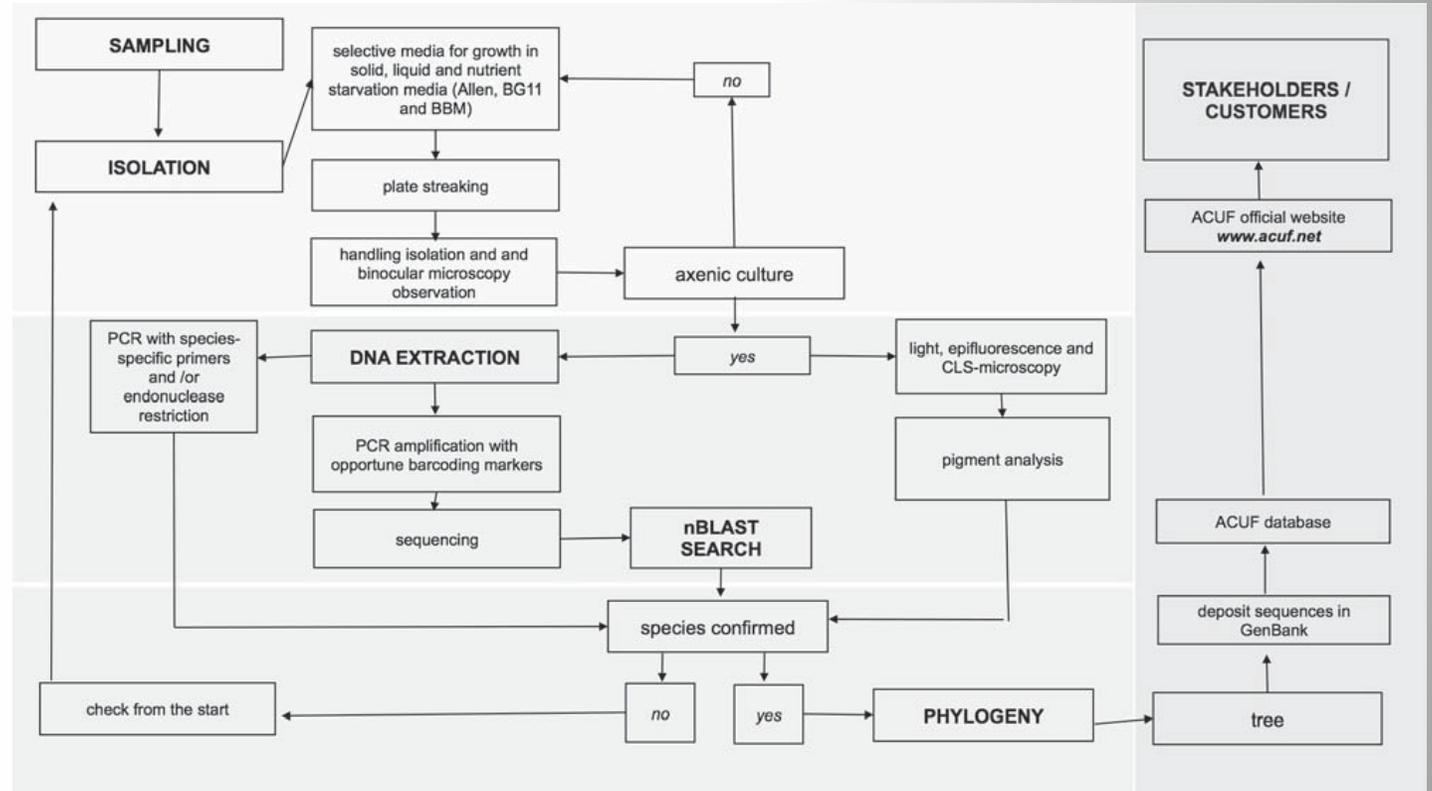


2024 New Mexico USA



Strain isolation procedures

The bulk of original isolates collected in 1970's and 1980's constitutes a historical resource of ACUF: they were mainly isolated by serial dilutions. Later on, other isolation techniques were applied, as single cells picking by micropipettes or driven by FACS.

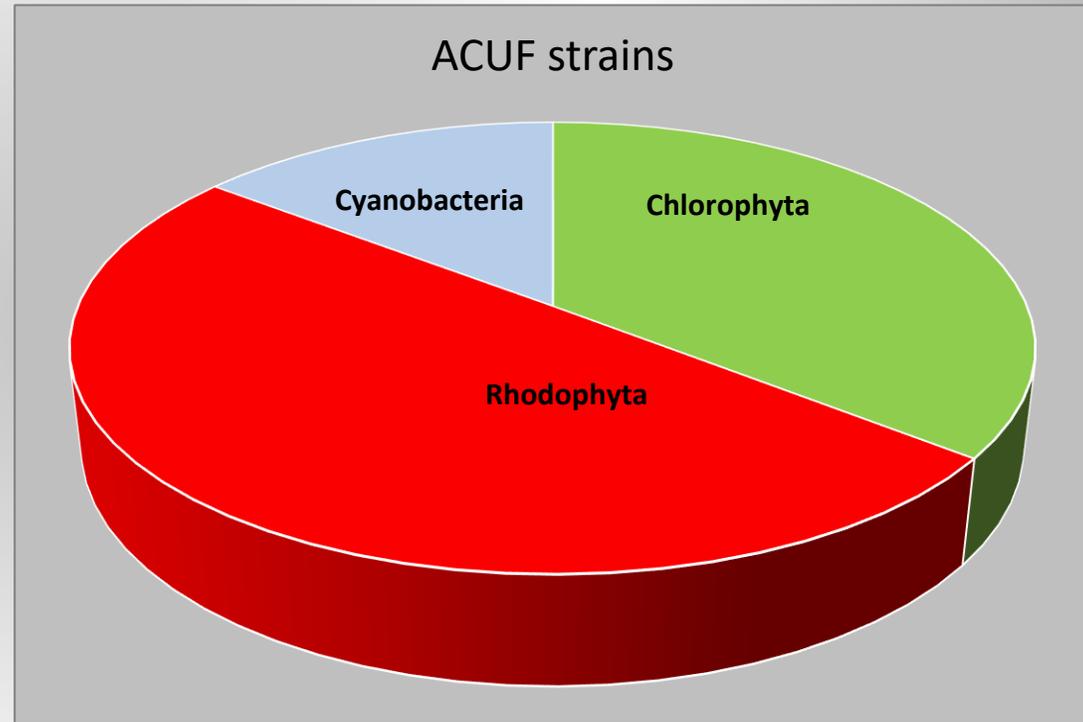


ACUF

ACUF presently maintains about 1000 strains

At least 3/4 of the strains kept in the collection are tolerant to one or more extreme conditions (low pH, temperature, drought).

ACUF main mission is to preserve and study the microbial biodiversity of extreme environments



Facilities and culture maintenance

- Most of ACUF strains are maintained in glass tubes at a range temperatures between 22°C and 24°C with various solid culture media as BG- 11, BBM and Allen (using Agar as solidifying agent).
- Few strains are maintained in liquid cultures at a temperature of 18°C. Cultures grow at an irradiance of 30 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ with a 16:8 h light-dark cycle.
- Under these conditions, transfers are made routinely every 2-3 months.





Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy

Area ESFRI “Health and Food”, granted by the European
Commission – NextGenerationEU

Code N° IR00000005

ACUF as a SUS-MIRRI partner

Cryopreservation facility

- Part of ACUF strains is also preserved in the cryopreservation facility, recently implemented thanks to funding from SUS-MIRRI project.
- All the laboratory tools and materials are stored in the cryopreservation facility.
- This facility includes:
 - 3 -80°C freezers
 - 1 -150°C freezer
 - 2 liophylizers
 - 1 CryoMed Controlled Rate Freezer
 - 3 Liquid nitrogen cell cryopreservation dewars



Not one
Galdieria
species, but (at
least) seven!

How to maintain and preserve
Galdieria strains belonging to
different species?

Developing tailored
cryopreservation protocols

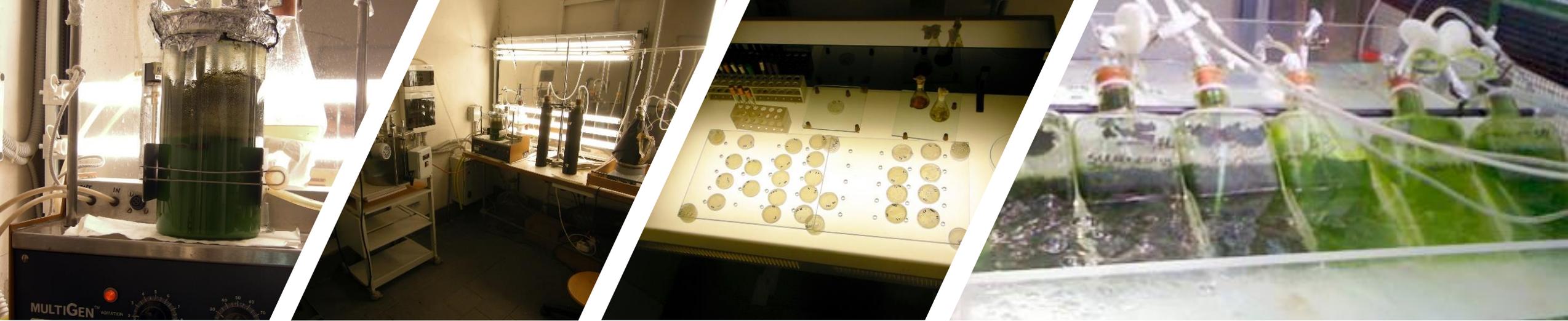




Biomass production facilities

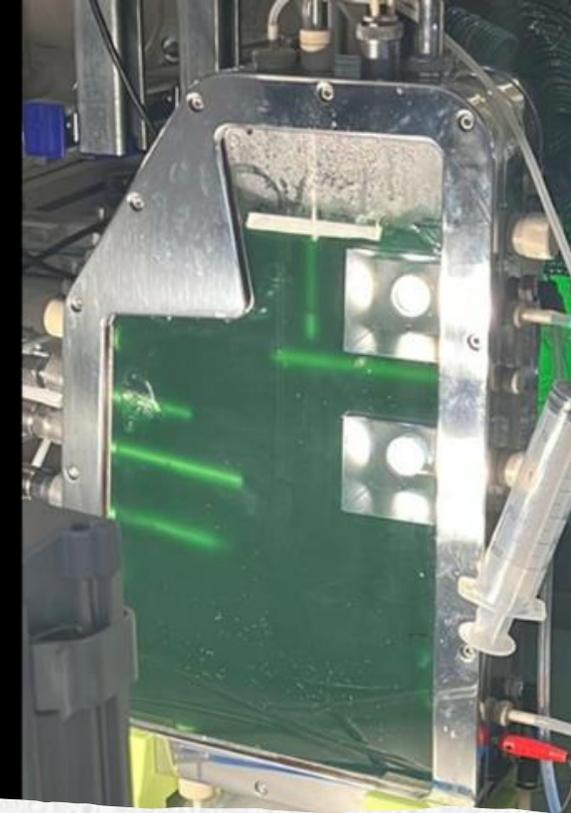
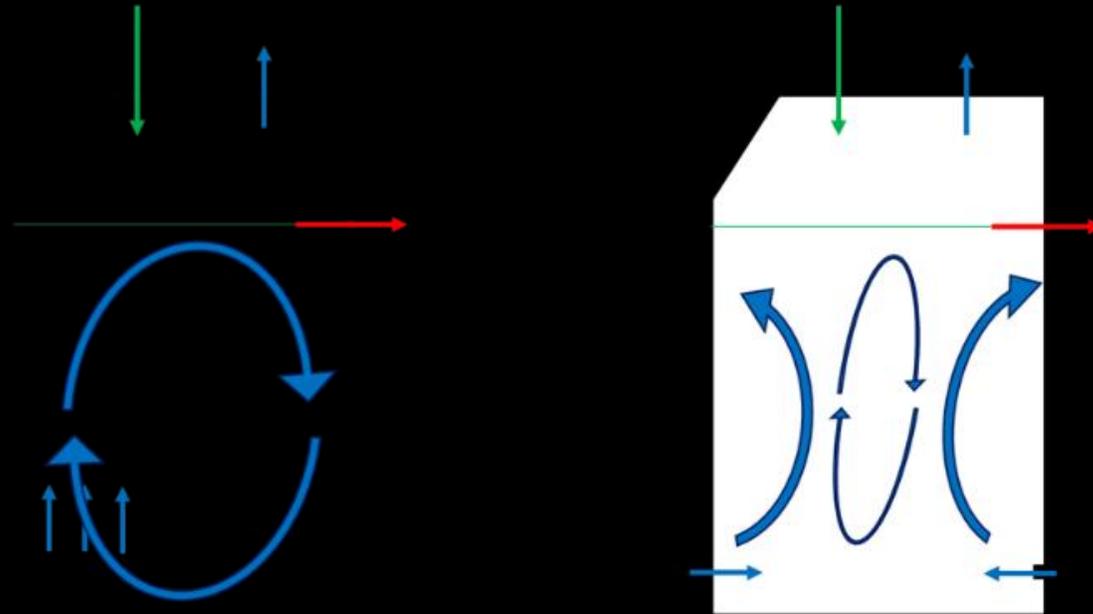
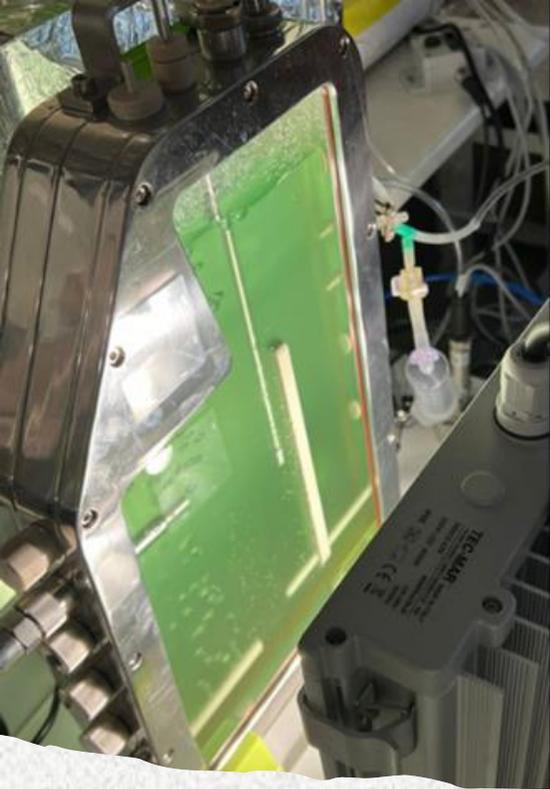
- For producing large volumes of cultures, ACUF is provided with growth chambers and different types of photobioreactors





Research at ACUF
extremophiles and extreme
tolerant strains as
biotechnological tools

Exploiting the potential of
Galdieria for biotechnological
applications: culturomics
approaches



Design and model of a biorefinery to fully exploit protein and sugar fractions from the extremophile microalga *Galdieria sulphuraria* (Marra et al. 2024)

- Modelling *Galdieria* growth and productivity in flat bioreactors

Exploring the biotechnological potential of *G. sulphuraria* strains from Ansanto valley (Italy), the largest natural emission of low temperature CO₂ rich gases, from non-volcanic environment, ever measured in the Earth (Chiodini et al. 2010)

- Cocultures of bacteria and *G. sulphuraria* isolated from samples collected along Ansanto Valley for carbon capture.





Mass cultures of extreme-tolerant strains in aquaculture

- *Viridiella fridericana*
- Dominik Refardt - ZHAW School of Life Sciences and Facility Management
Institute of Natural Resource Sciences- Switzerland



Horizon 2020
European Union funding
for Research & Innovation



Algae4IBD

Health from aquatic resources

From Nature to Bedside- Algae Based Bio Compound for Prevention and Treatment of Inflammation, Pain and IBD

130 extremophilic and extreme-tolerant ACUF strains are under investigation in search of active compounds



ACUF



From ACUF to MACUF

- MACUF - Microbiome and Algae Collection at the University Federico II of Naples

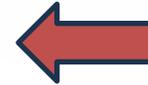
MICROBIOMES from extreme environments:

- Sampling
- Characterization
- Maintenance
- Growth

Extremophilic microbiomes: sampling and characterization

SOP (standard operation procedure) establishment:

SOP for GEOTHERMAL SPRINGS SEDIMENTS SAMPLING



SAMPLING

CHARACTERIZATION

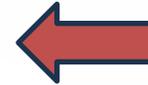


SOP (standard operation procedure) establishment:

SOP for MICROBIOME DNA EXTRACTION

Extremophilic microbiomes: maintenance and growth

Cryopreservation under different conditions



MAINTENANCE

GROWTH



Winogradski Columns

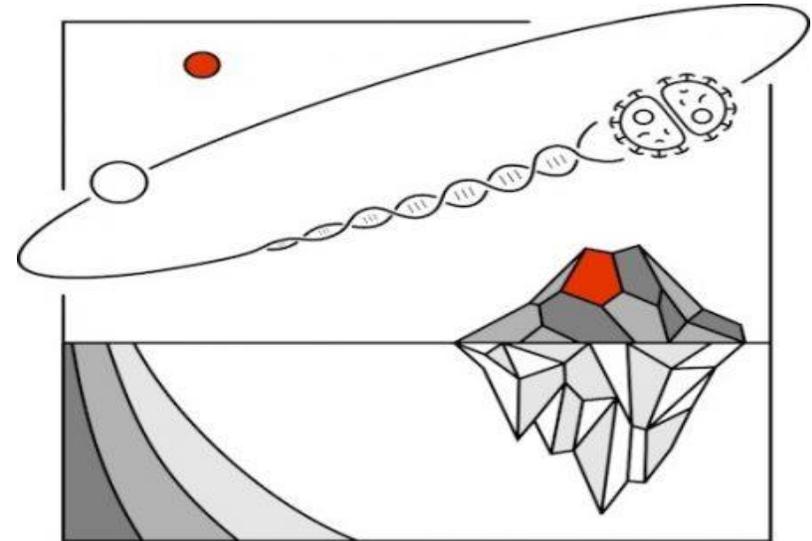
Teaching and outreaching at ACUF

ACUF participates to the 2-year international master course **Biology of Extreme Environments** (BIOEXTREME)

[Department of Biology, University Federico II of Naples, Italy]

ACUF has entered an agreement with the Società dei Naturalisti in Naples for the digitalization of travel booklets, photos and lab data sheets of Professor Roberto Taddei.

This material will be used for an exhibition on the history of the collection, to be released on the web in 2025.



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ACUF

