MICROBIOLOGY OF EXTREME ENVIRONMENTS

EXTREMOPHILES AND THEIR CONTRIBUTION TO SOCIETY

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BIOLOGY OF EXTREME ENVIRONMENTS

DIPARTIMENTO DI BIOLOGIA

WHY STUDYING EXTREMEOPHILES?

Why studying extremophiles?

We can study extremophiles for several reasons:

- As model to understand the origin and evolution of Life on Earth
- As model to understanding basic Life principles
- As model in the search for extraterrestrial Life
- For their contribution to our planet functioning
- For their biotechnological applications

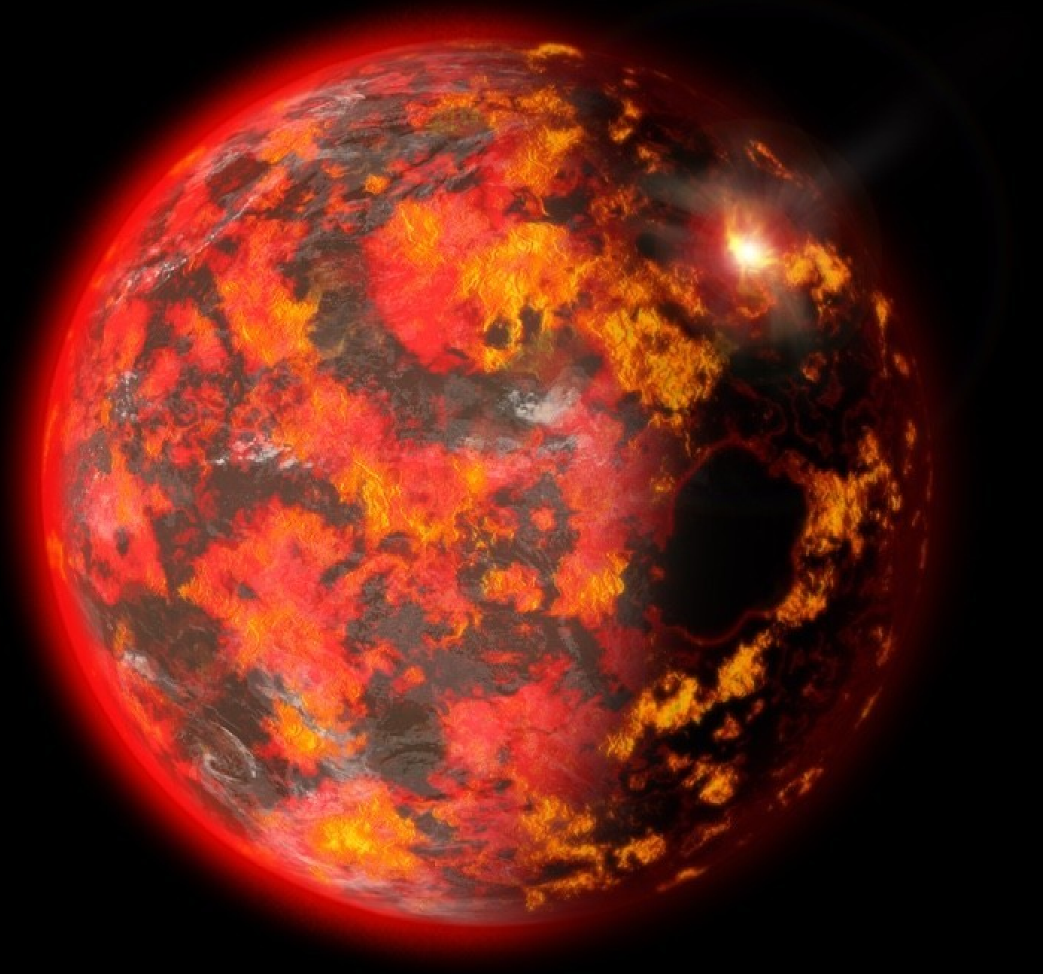
As model to understand the origin and evolution of Life on Earth:

What were the environmental conditions early during the first billion year of history of Earth?

As model to understand the origin and evolution of Life on Earth:

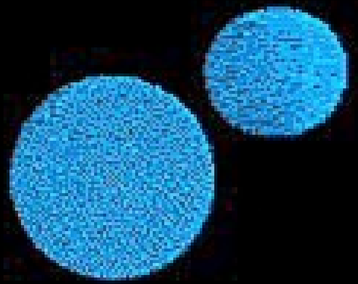
As model to understand the origin and evolution of Life on Earth:

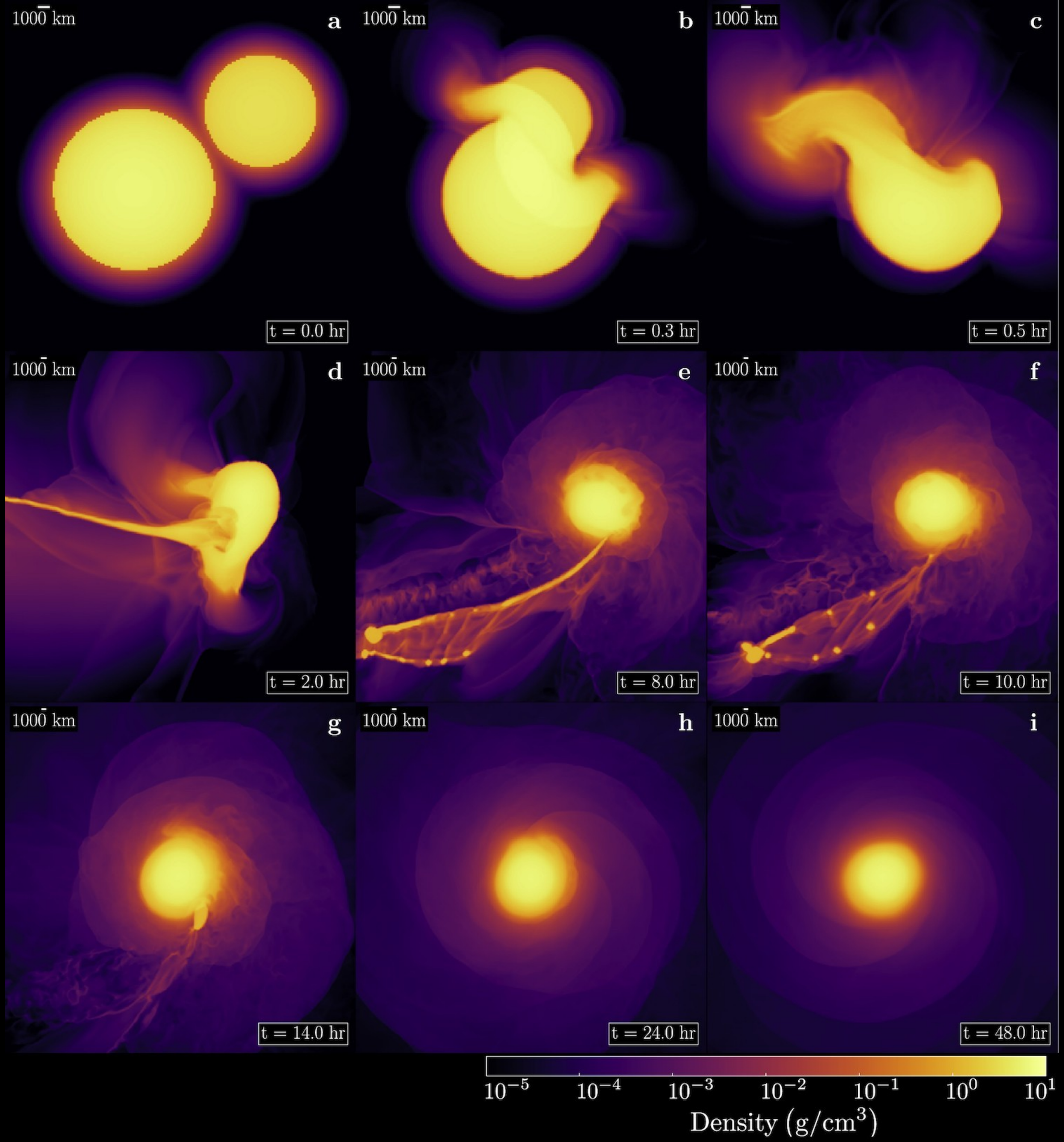
- *Accretion*
- *Self-compression*
- *Core differentiation*
- *Magma ocean*
- *First atmosphere*
- *First crust*
- *First ocean*
- *Plate tectonic*



Magma ocean

Moon forming impact

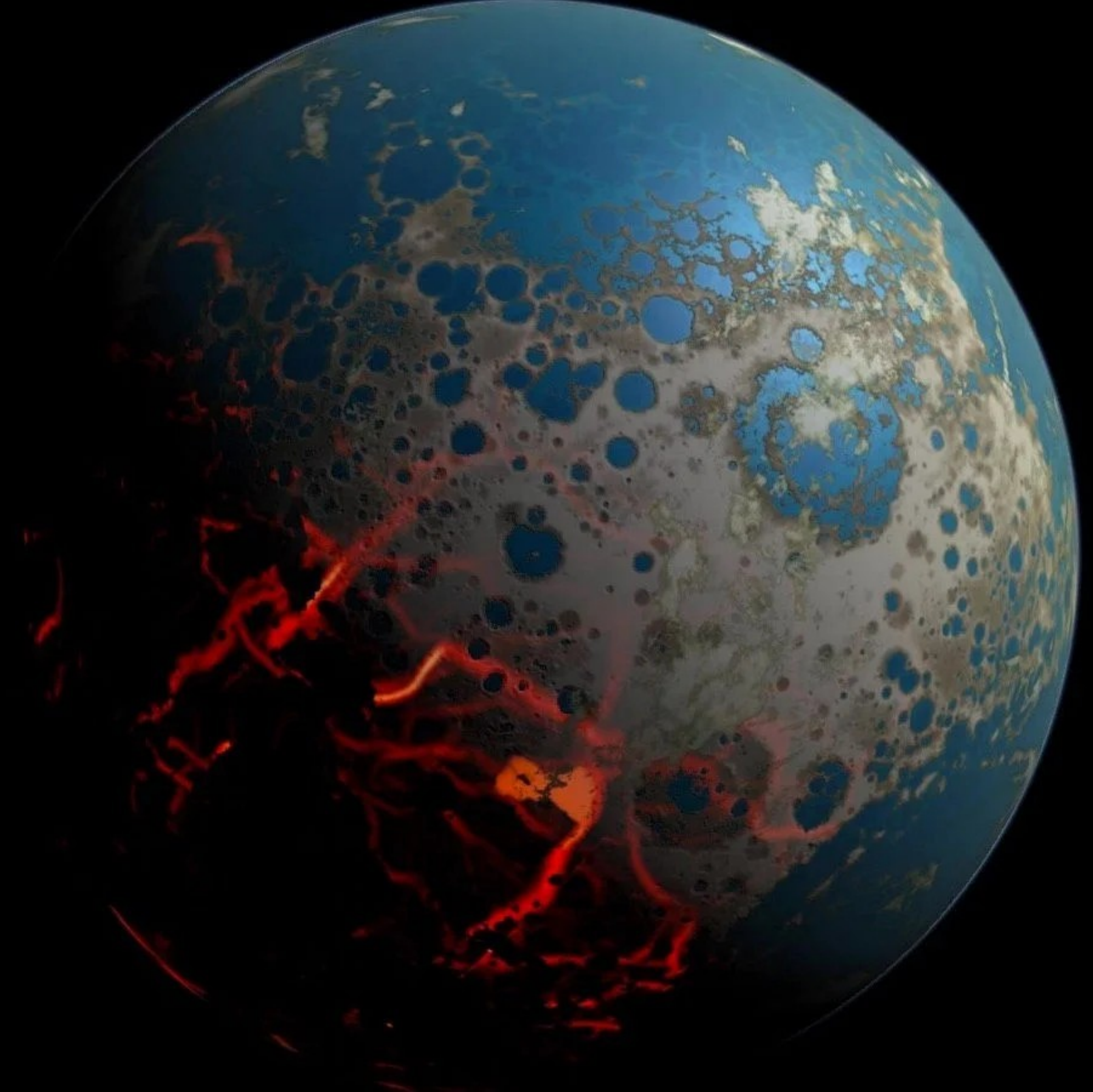




1000 km
a
t = 0.0 hr
1000 km
b
t = 0.3 hr
1000 km
c
t = 0.5 hr
1000 km
d
t = 2.0 hr
1000 km
e
t = 8.0 hr
1000 km
f
t = 10.0 hr
1000 km
g
t = 14.0 hr
1000 km
h
t = 24.0 hr
1000 km
i
t = 48.0 hr
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1}
Density (g/cm^3)

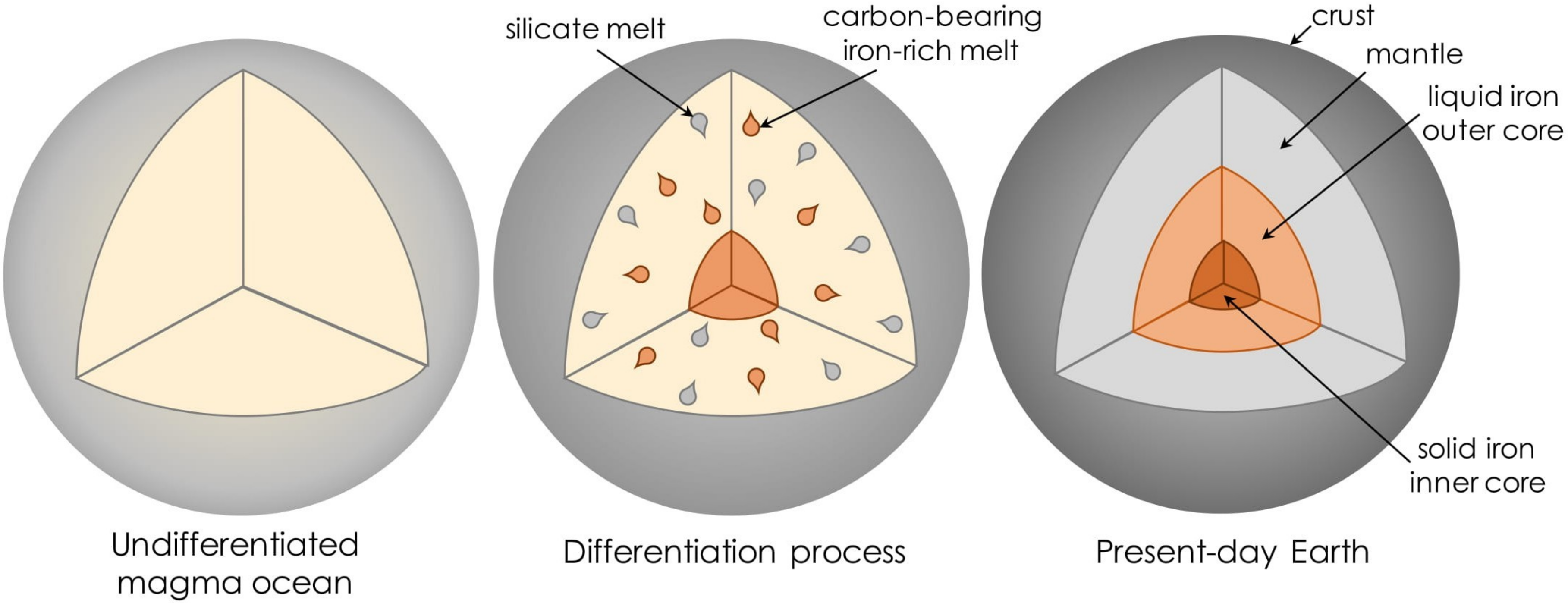
A closer Moon and shorter days


The faint young Sun paradox



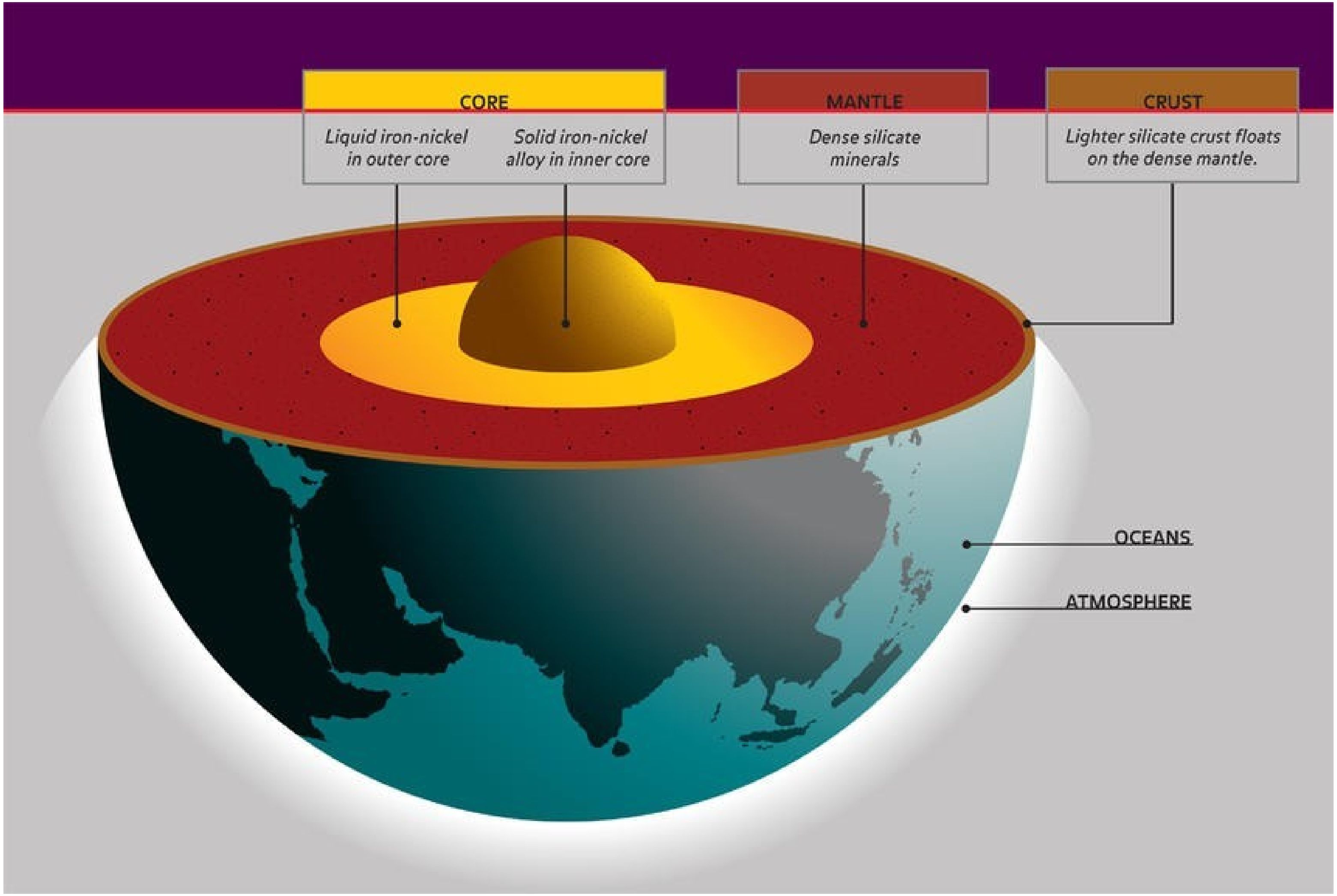
The first crust

As model to understand the origin and evolution of Life on Earth:



Crust differentiation

As model to understand the origin and evolution of Life on Earth:



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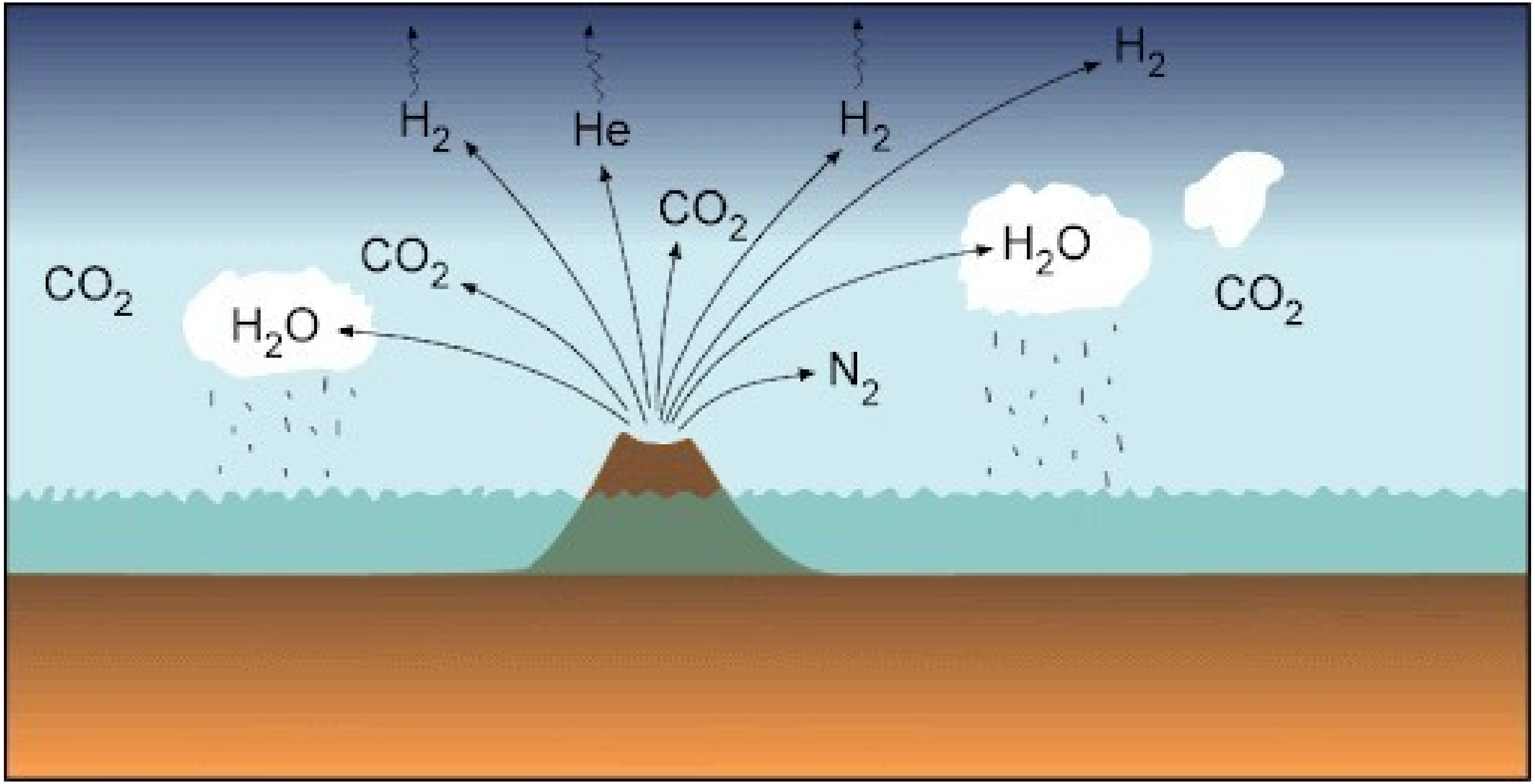
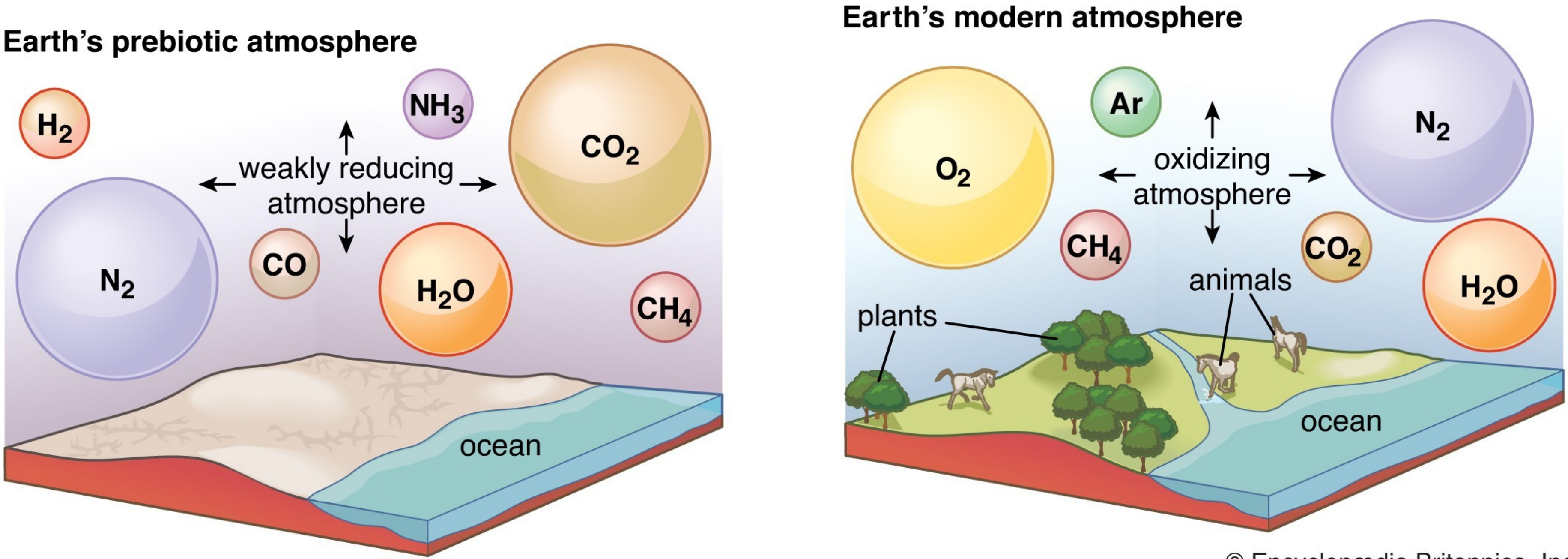
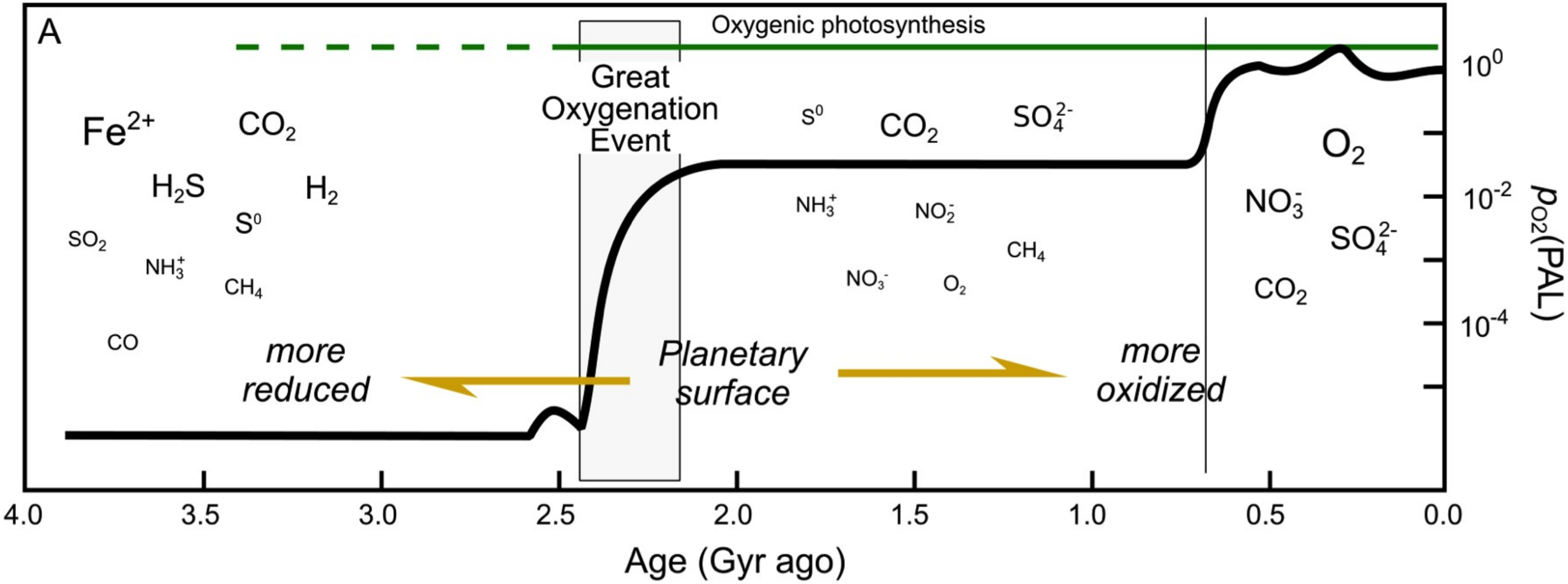


First atmosphere and first ocean

As model to understand the origin and evolution of Life on Earth:



As model to understand the origin and evolution of Life on Earth:



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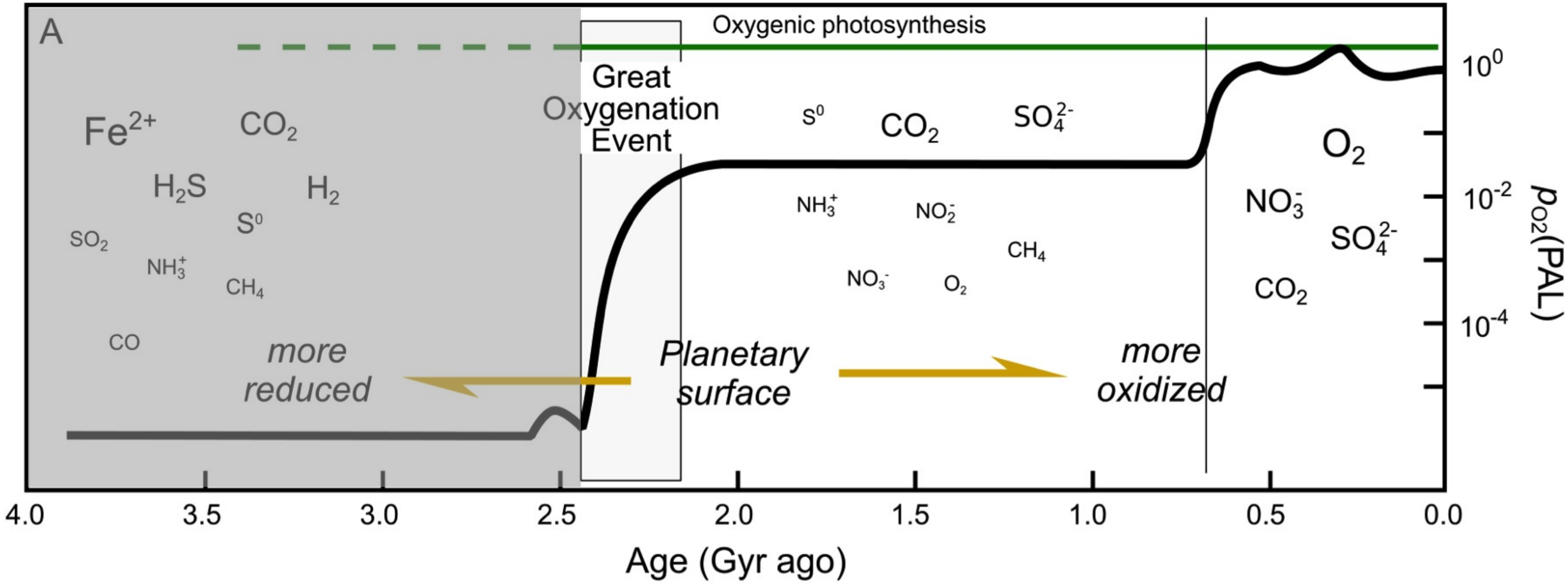

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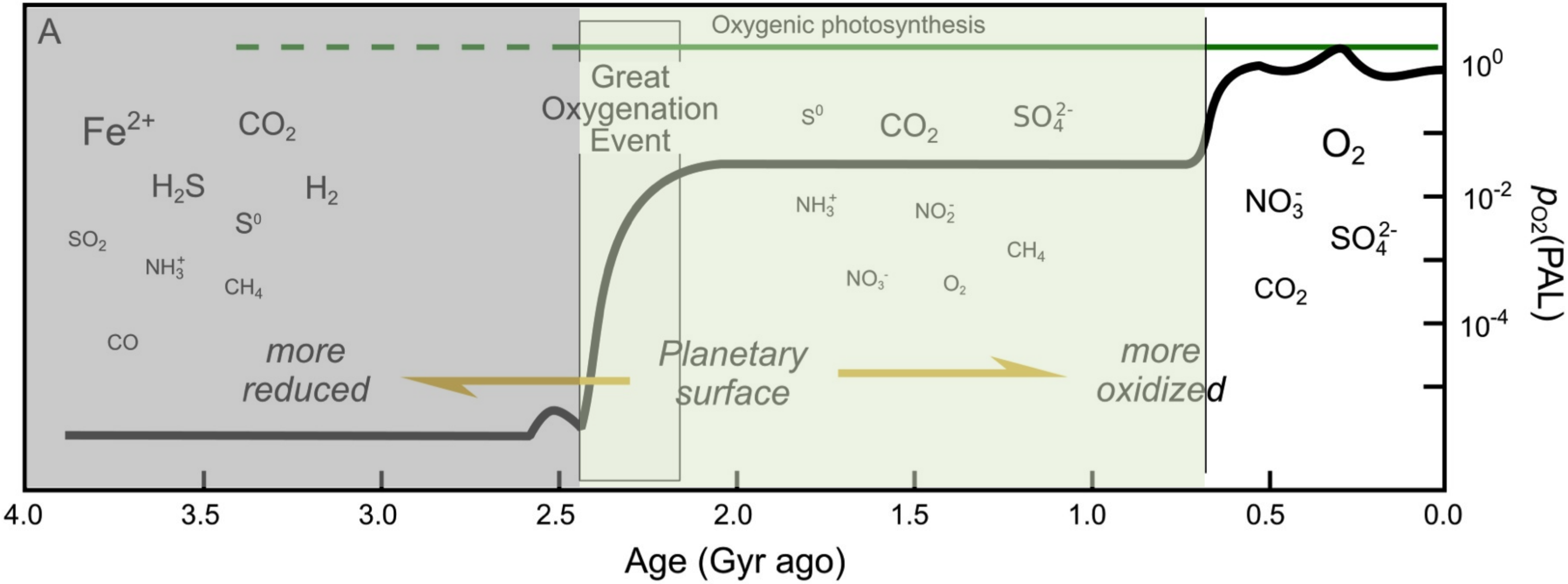


As model to understand the origin and evolution of Life on Earth:

Fe^{2+} CO_2

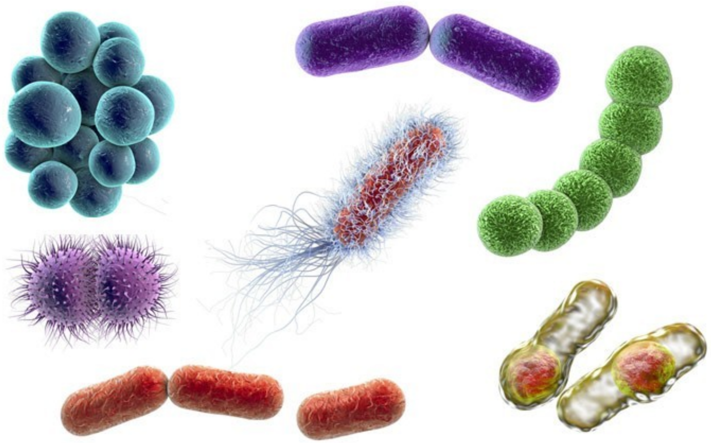
H_2S H_2

S^0

SO_2

NH_3^+

CH_4



As model to understand the origin and evolution of Life on Earth:

Fe^{2+} CO_2

H_2S H_2

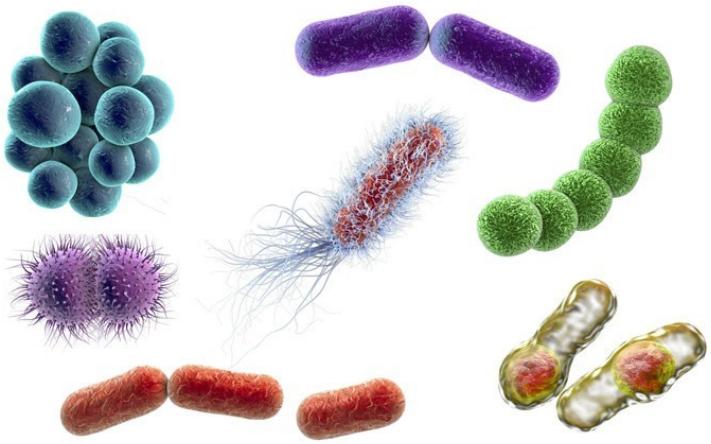
S^0

SO_2 NH_3^+ CH_4

Methanogenesis?

Ammonia oxidation?

Sulfur reduction?



Anoxygenic photosynthesis?

Hydrogen oxidation?

Iron oxidation?

As model to understanding basic Life principles

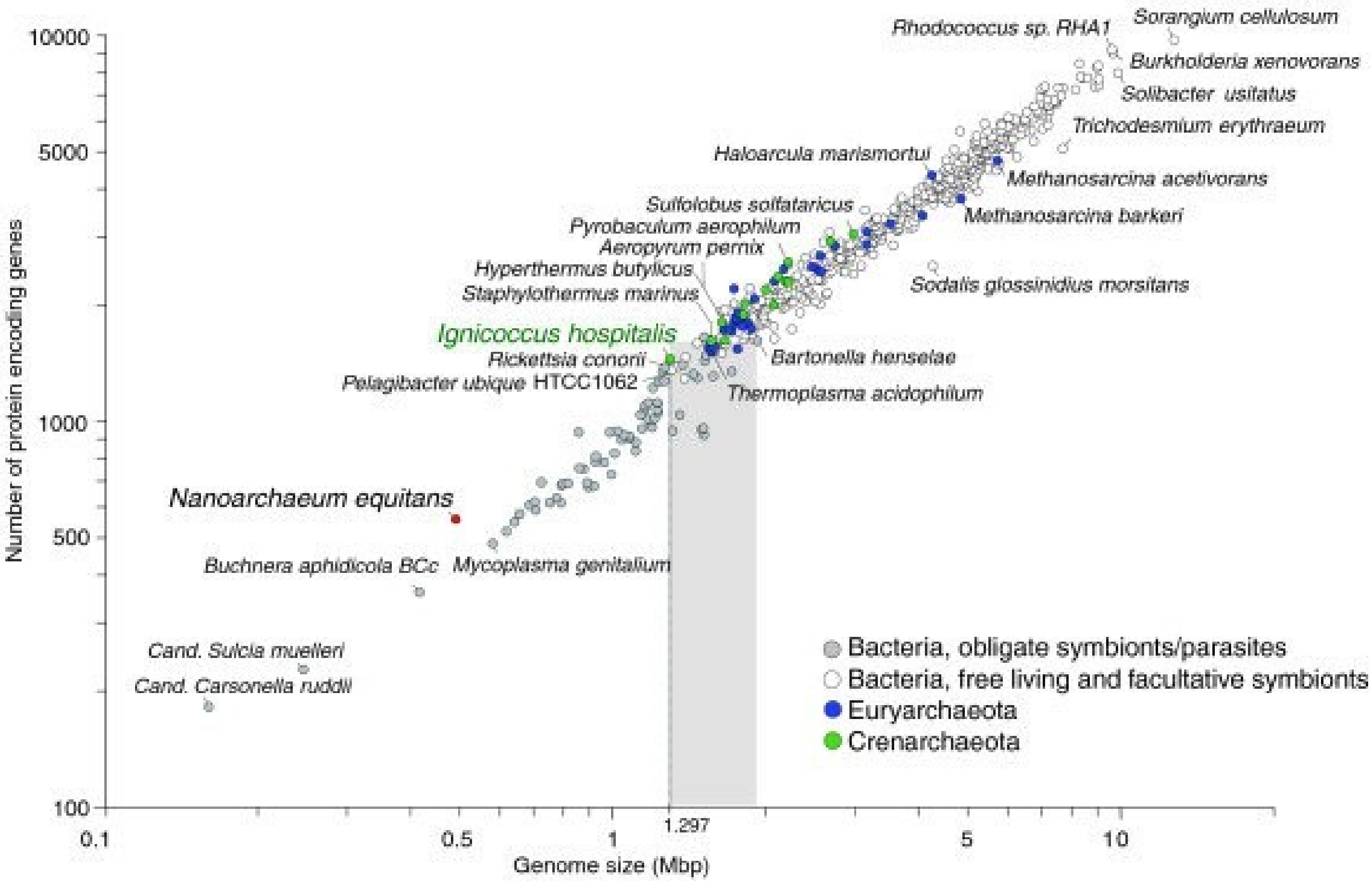
Table 1.
Ecological requirements for life

Requirement	**Occurrence in the Solar System**
Energy	Common
Predominately light	Photosynthesis at 100 AU light levels
Chemical energy	e.g., $H_2 + CO_2 \rightarrow CH_4 + H_2O$
Carbon	Common as CO_2 and CH_4
Liquid water	Rare, only on Earth for certain
N,P, S, Na, and other elements	Likely to be common

- From ref. 2.

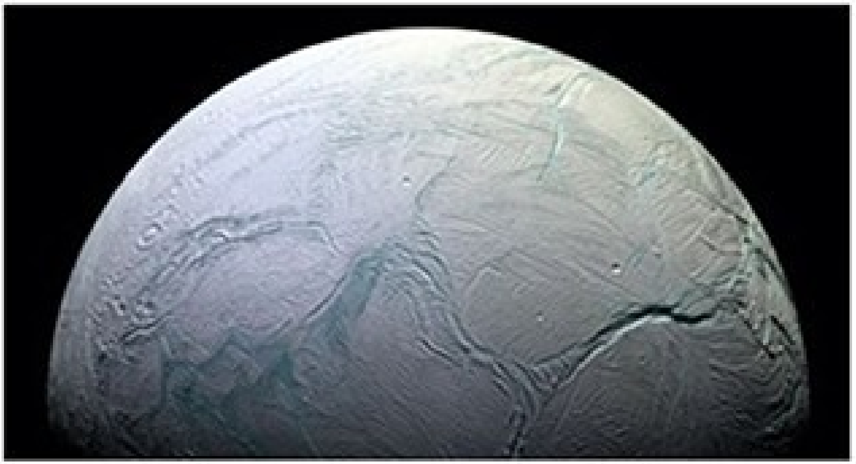
McKay 2014 PNAS

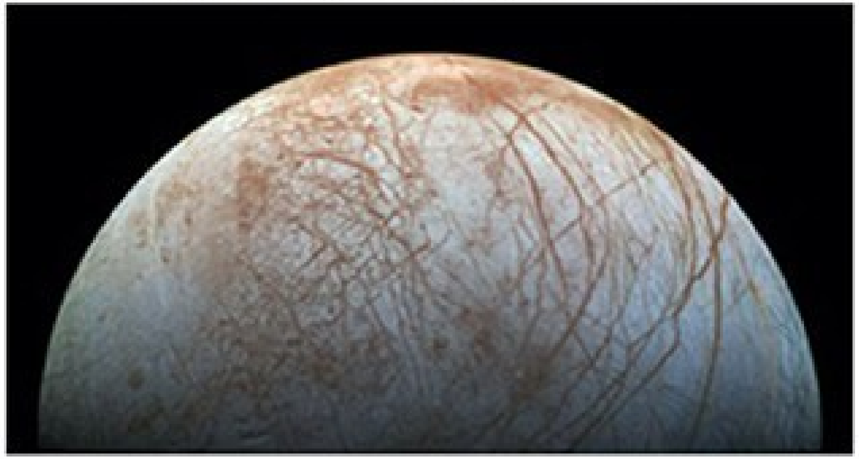
As model to understanding basic Life principles

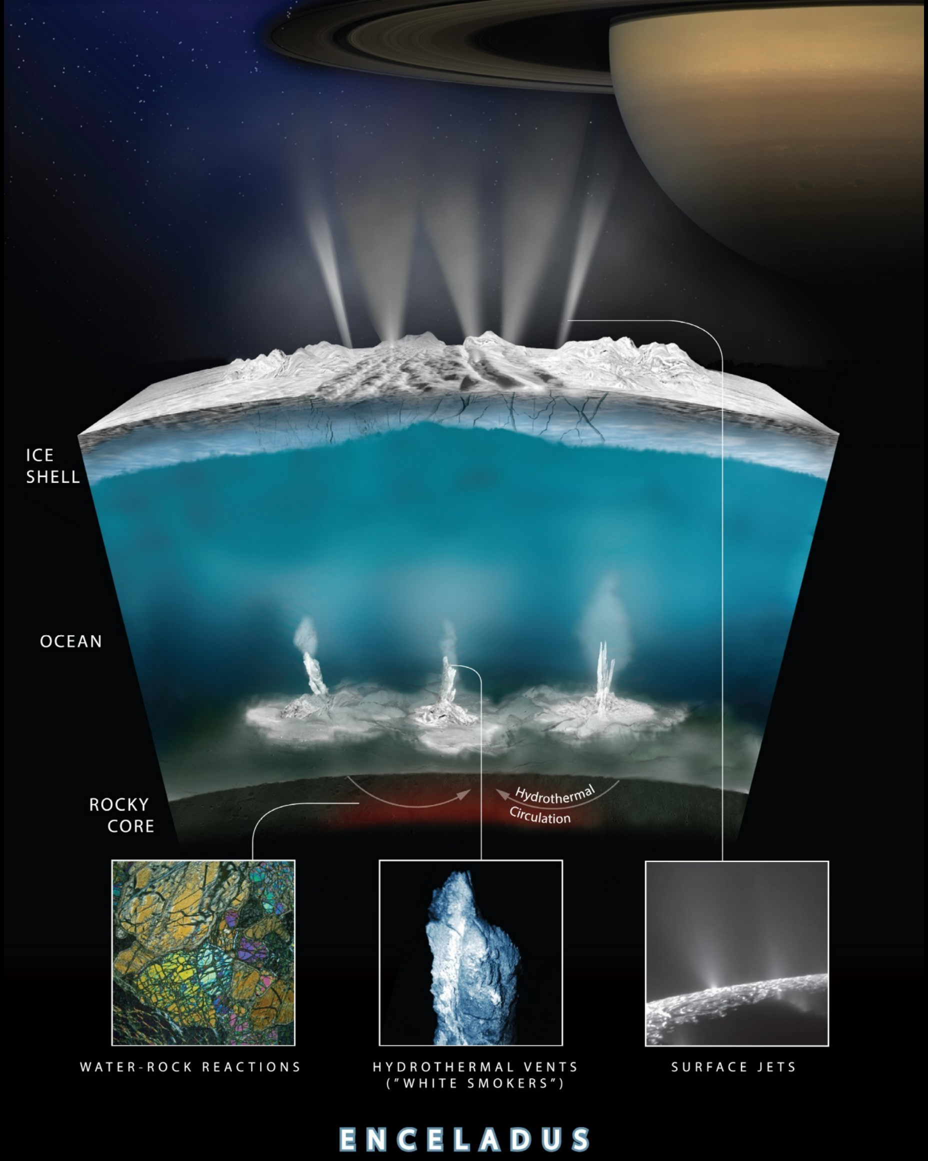


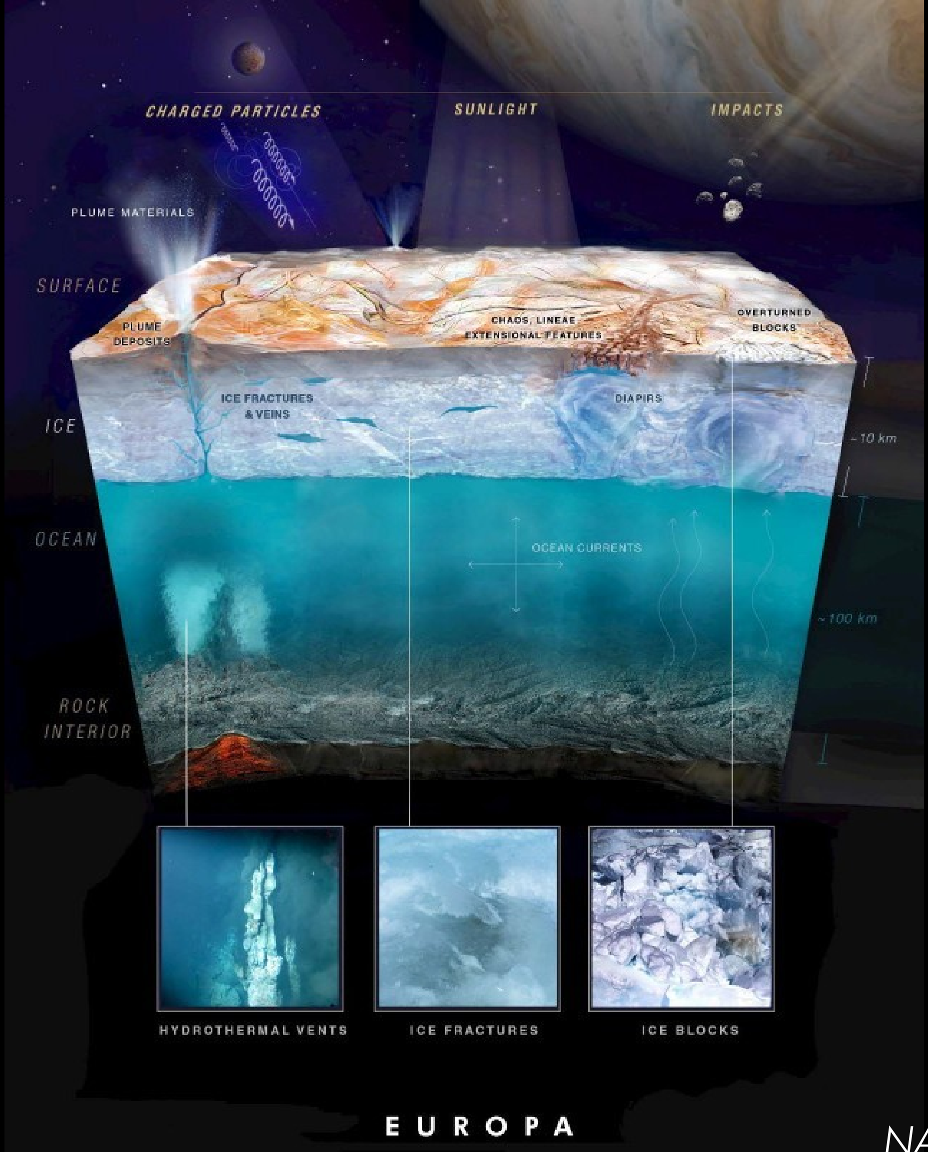
Podar et al 2008 Genome Biology

As model in the search for extraterrestrial Life



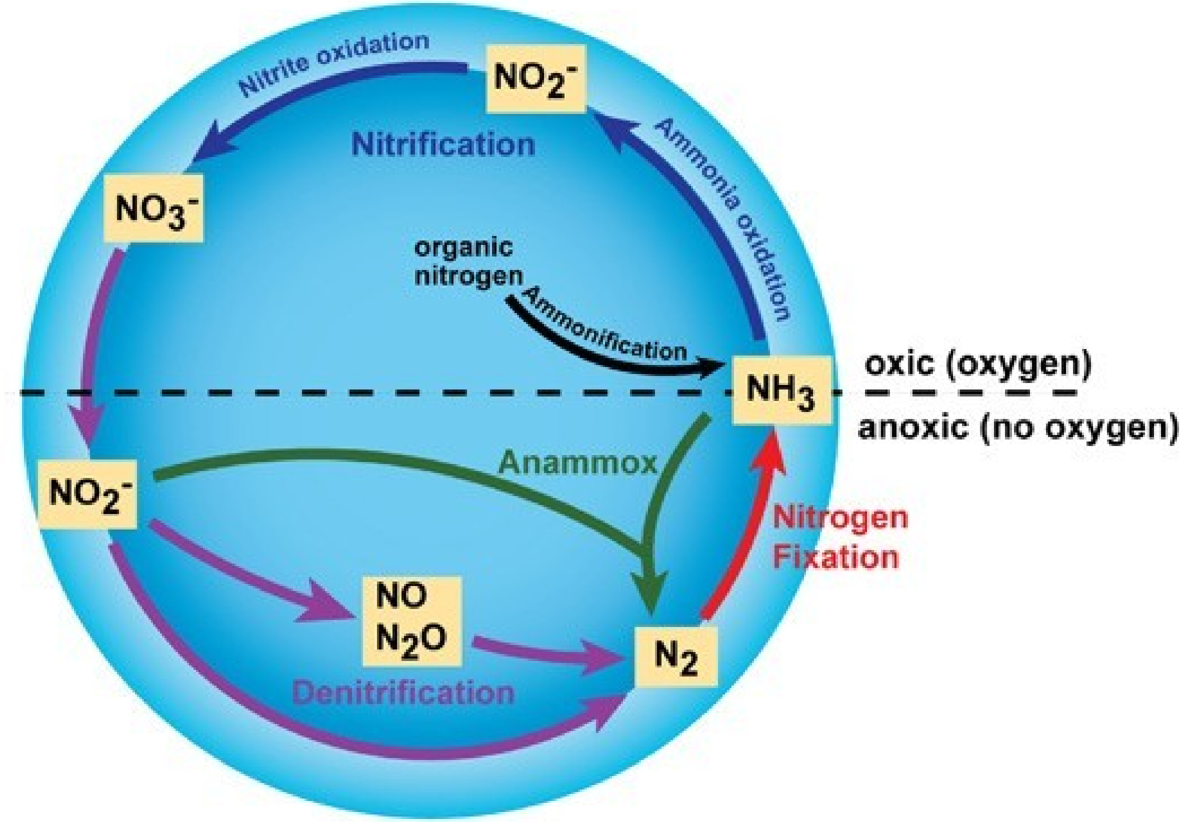



ICE
SHELL
OCEAN
ROCKY
CORE
Hydrothermal
Circulation
WATER-ROCK REACTIONS
HYDROTHERMAL VENTS
("WHITE SMOKERS")
SURFACE JETS
ENCELADUS


CHARGED PARTICLES
SUNLIGHT
IMPACTS
PLUME MATERIALS
SURFACE
PLUME
DEPOSITS
CHAOS, LINEAE
EXTENSIONAL FEATURES
OVERTURNED
BLOCKS
ICE
ICE FRACTURES
& VEINS
DIAPIRS
~10 km
OCEAN
OCEAN CURRENTS
~100 km
ROCK
INTERIOR
HYDROTHERMAL VENTS
ICE FRACTURES
ICE BLOCKS
EUROPA

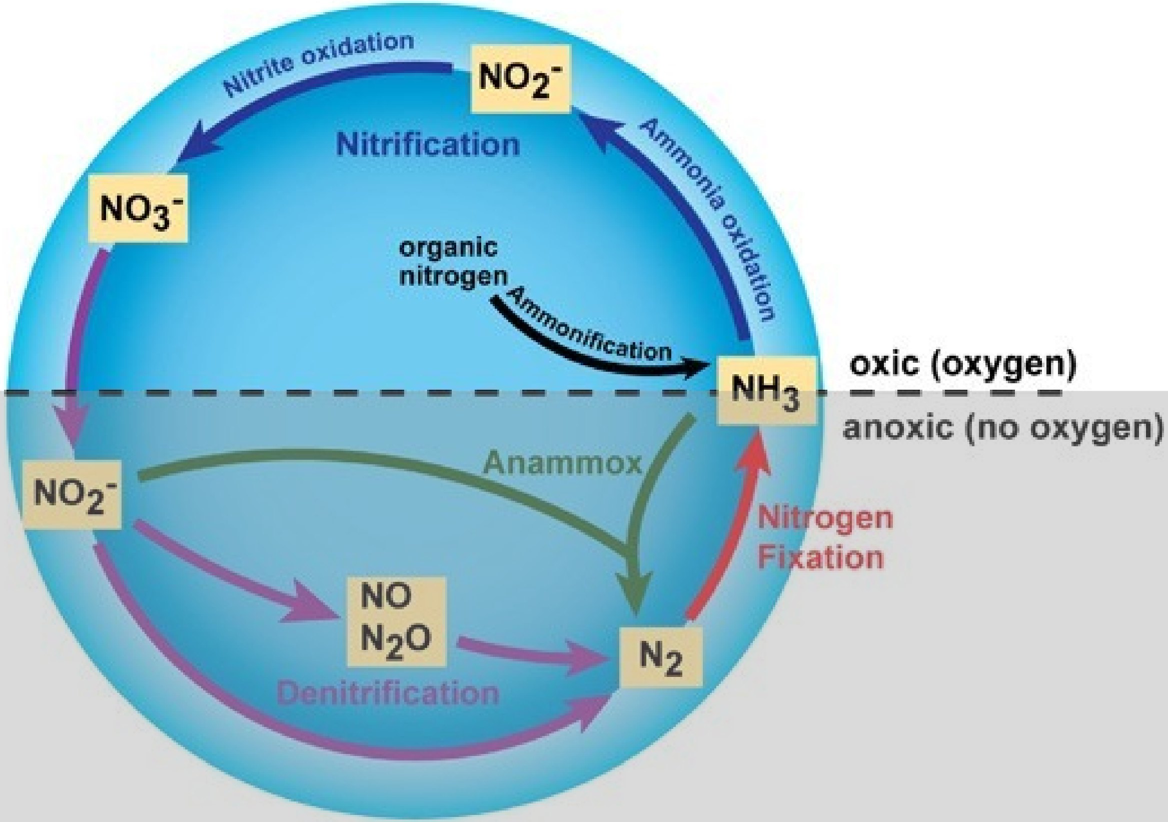
NASA

For their contribution to our planet functioning



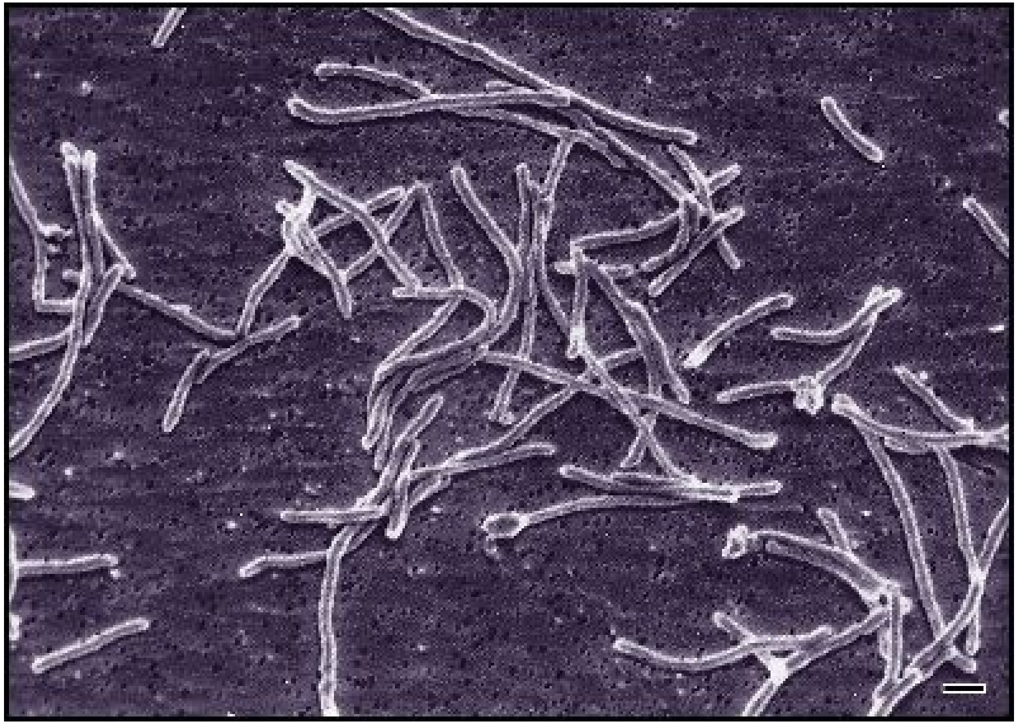
2010 Nature Education

For their contribution to our planet functioning



2010 Nature Education

For their biotechnological applications



Thermus aquaticus

For their biotechnological applications

JOURNAL OF BACTERIOLOGY, Aug. 1969, p. 289–297 Vol. 98, No. 1
 Printed in U.S.A.

Thermus aquaticus gen. n. and sp. n., a Non-sporulating Extreme Thermophile

THOMAS D. BROCK AND HUDSON FREEZE

Department of Microbiology, Indiana University, Bloomington, Indiana 47401

Received for publication 15 January 1969

The isolation of a new thermophilic bacterium, *Thermus aquaticus* gen. n. and sp. n., is described. Successful enrichment requires incubation at 70 to 75 C, and the use of nutrient media relatively dilute with respect to the organic components. Strains of *T. aquaticus* have been isolated from a variety of thermal springs in Yellowstone National Park and from a thermal spring in California. The organism has also been isolated from man-made thermal habitats, such as hot tap water, in geographical locations quite distant from thermal springs. Isolates of *T. aquaticus* are gram-negative nonsporulating nonmotile rods which frequently form long filaments at supraoptimal temperatures or in the stationary phase. All isolates form a yellow cellular pigment, probably a carotenoid. A characteristic structure formed by all isolates is a large sphere, considerably larger than a spheroplast. These large spheres, as well as lysozyme-induced spheroplasts, are resistant to osmotic lysis. Deoxyribonucleic acid base compositions of four strains were determined by CsCl density gradient ultracentrifugation and found to be between 65.4 and 67.4 moles per cent guanine plus cytosine. The growth of all isolates tested is inhibited by fairly low concentrations of cycloserine, streptomycin, penicillin, novobiocin, tetracycline, and chloramphenicol. Nutritional studies on one strain showed that it did not require vitamins or amino acids, although growth was considerably faster in enriched than in synthetic medium. Several sugars and organic acids served

For their biotechnological applications

1967 Brooks explained: "Bacteria are able to grow [...] at any temperature at which there is liquid water, even in pools which are above the boiling point."

In 1976 the thermostable enzyme DNA polymerase was first isolated from Thermus aquaticus

In 1983 the Taq enzyme became the cornerstone of Kary Mullis invention of the Polymerase Chain Reaction (PCR) for which he won the Noble Prize in 1993

Thermostable polymerase enzymes derived from Taq are now an industry worth ca. 400 Million euro/year

PCR and polymerases are key technologies for biological, genetics, biomedicine and biotechnology research, a global business of hundred billions of euro

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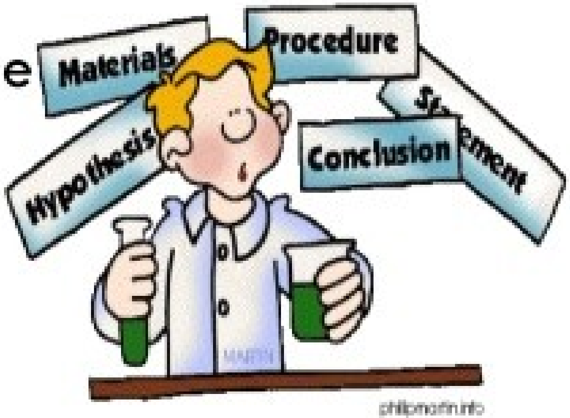
Basic or applied reasearch?

For their biotechnological applications

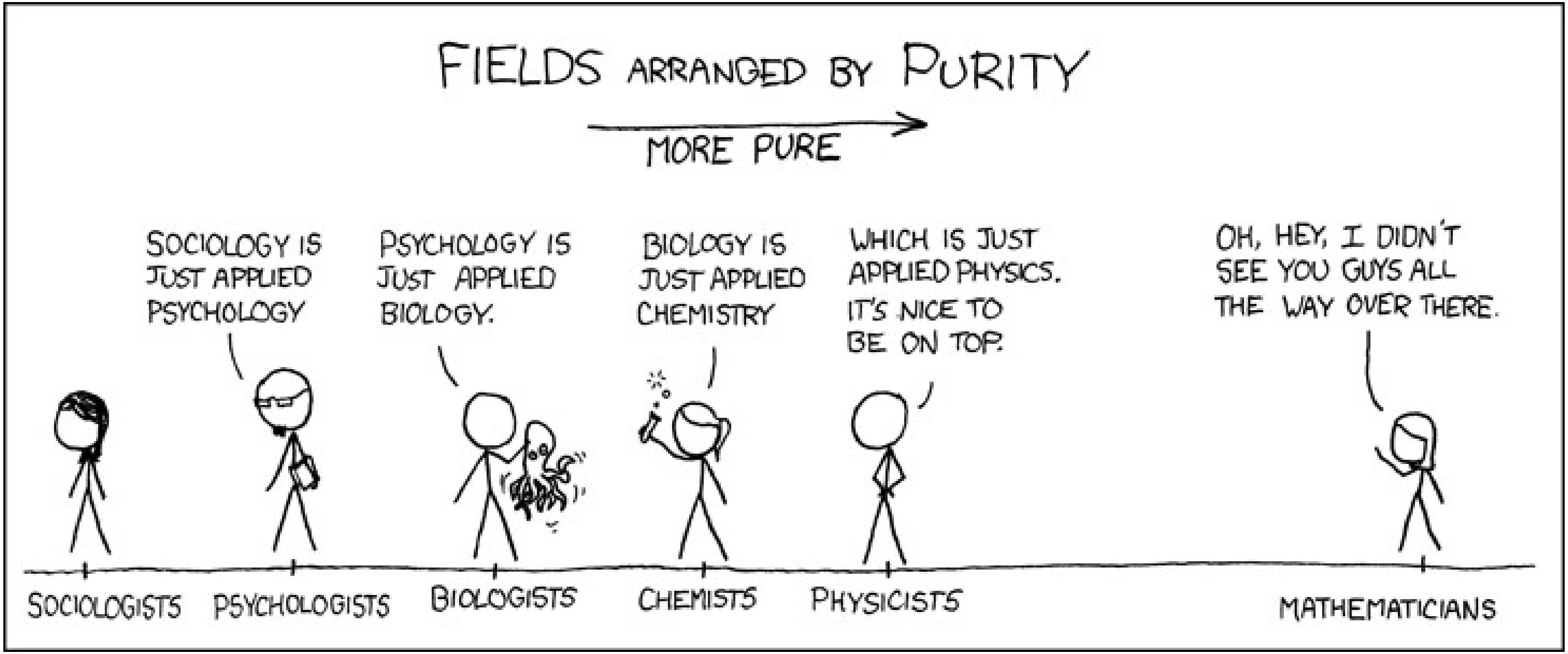
BASIC AND APPLIED SCIENCE

- Basic Science seeks to expand knowledge
- Aim is to satisfy human thought



- Applied Science uses basic science to solve real-world problems.
- Researchers utilize widespread information – theories and hypothesis of basic science to arrive at a solution

For their biotechnological applications



https://xkcd.com/

BASIC APPLIED

BASIC → APPLIED

BASIC ⟷ APPLIED

The use of basic science

C.H. Llewellyn Smith

https://bit.ly/2CtWzvz

RIP: The Basic/Applied Research Dichotomy

Venkatesh Narayanamurti, Tolu Odumosu, Lee Vinsel

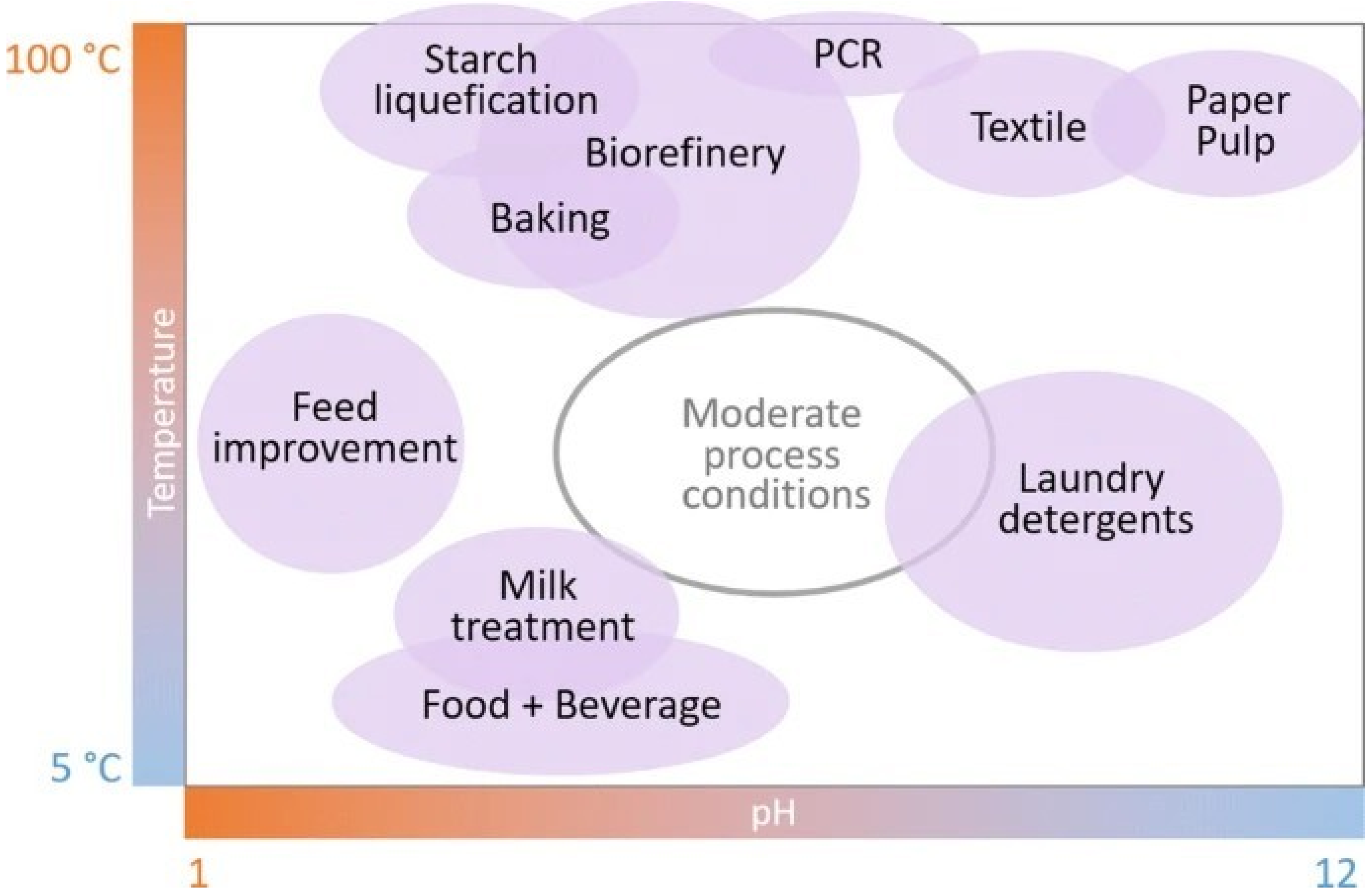
https://bit.ly/2TbNpcx

Why the distinction between basic (theoretical) and applied (practical) research is important in the politics of science

Nils Roll-Hansen

https://bit.ly/2CsvOb0

For their biotechnological applications



Schröder et al 2020 ChemTexts


Industrial enzymes


Industrial enzymes


Bioactive compounds


Industrial enzymes

Bioactive compounds

Bioremediation


Industrial enzymes


Bioactive compounds


Bioremediation


Crop production


Industrial enzymes

Bioactive compounds

Bioremediation

Crop production

Biogas and Biofuels


Industrial enzymes


Bioactive compounds


Bioremediation


Crop production


Biogas and Biofuels


Underground fuel storage

Industrial enzymes

Bioactive compounds

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Geoengineering

A BRIEF HISTORY OF THERMOPHILES

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HOW HOT IS TOO HOT?

PNAS
Proceedings of the
National Academy of Sciences
of the United States of America

RESEARCH ARTICLE

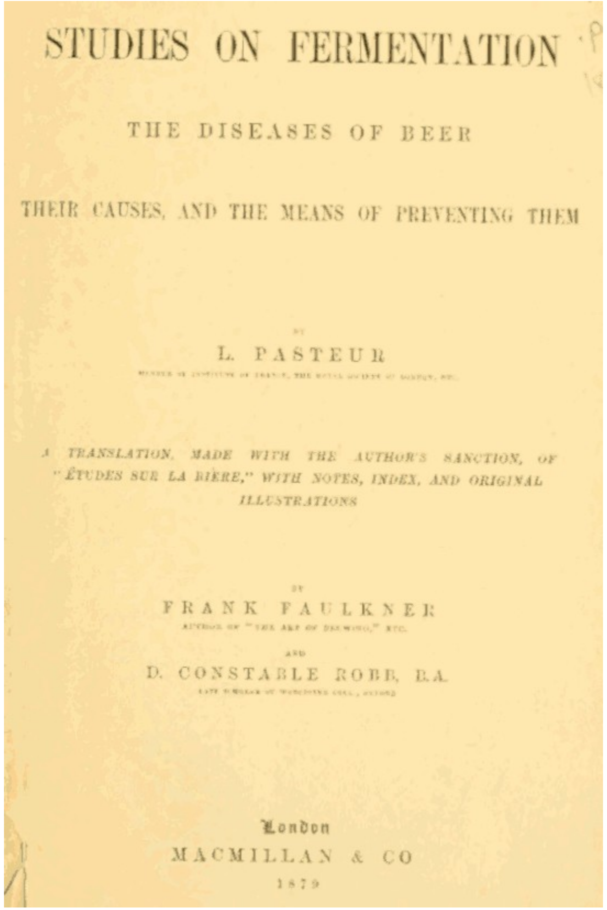
Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation

Ken Takai, Kentaro Nakamura, Tomohiro Toki, Urumu Tsunogai, Masayuki Miyazaki, Junichi Miyazaki, Hisako Hirayama, Satoshi Nakagawa, Takuro Nunoura, and Koki Horikoshi

PNAS August 5, 2008 105 (31) 10949-10954; https://doi.org/10.1073/pnas.0712334105

Edited by James M. Tiedje, Michigan State University, East Lansing, MI, and approved May 12, 2008 (received for review January 6, 2008)

A (very) brief history of thermophiles



STUDIES ON FERMENTATION

THE DISEASES OF BEER

THEIR CAUSES, AND THE MEANS OF PREVENTING THEM

L. PASTEUR

A TRANSLATION, MADE WITH THE AUTHOR'S SANCTION, OF "ÉTUDES SUR LA BIÈRE," WITH NOTES, INDEX, AND ORIGINAL ILLUSTRATIONS

FRANK FAULKNER

AND

D. CONSTABLE ROBB, B.A.

London

MACMILLAN & CO

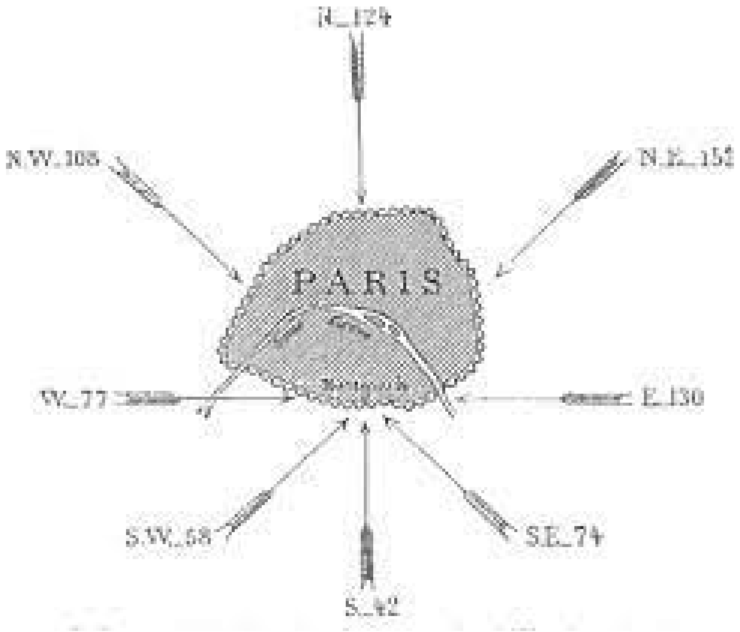
1879

At the dawn of microbiology, it had been generally accepted that vegetative cells of bacteria are safely killed at temperatures between 80 and 100 °C.

These observation were fundamental in the Germ theory of Louis Pasteur and the development of pasteurization ~(1860).

This lead to the generally accepted idea that life's limit were around 75-80 °C.

A (very) brief history of thermophiles



In 1881, Pierre Miquel while studying the microbiology of the Seine river, isolated bacteria that grew at 60-70 °C, with an upper temperature limit of 75 °C.

"It is curious," he wrote, "to see a living organism growing in a liquid medium where the hand is harshly burnt in a few seconds."

A (very) brief history of thermophiles

Science

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HOME > SCIENCE > VOL. 142, NO. 3597 > UPPER TEMPERATURE LIMIT OF LIFE

REPORT

Upper Temperature Limit of Life

ELLIS S. KEMPNER

SCIENCE • 6 Dec 1963 • Vol 142, Issue 3597 • pp. 1318-1319 • DOI: 10.1126/science.142.3597.1318

Abstract

Samples of microorganisms from the hot springs of Yellowstone National Park have been collected and tested for the ability to utilize radioactive phosphorus. No evidence for growth was found above 73°C.

By 1960s-'70s, some 80 years later, upper temperature limit for growth of microorganisms was believed to be ~70 °C.

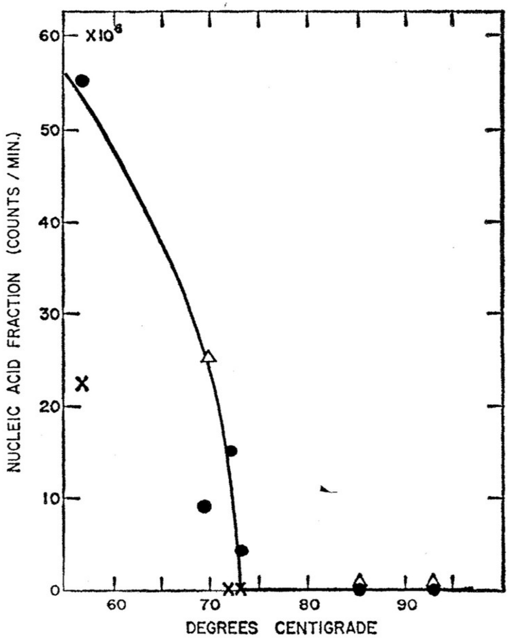
Most of the thermophilic bacteria that had been described by earlier researchers were members of a group called the spore-forming bacteria (because they produced heat-resistant spores), and no organisms had ever been isolated from high temperature geothermal environments up to that point.

Upper Temperature Limit of Life

Abstract. *Samples of microorganisms from the hot springs of Yellowstone National Park have been collected and tested for the ability to utilize radioactive phosphorus. No evidence for growth was found above 73°C.*

Survival of organisms under extreme conditions may depend on isolation of their internal environment from inordinate salt concentrations, *p*H, or even pressure. However, there would be no defense against high temperatures unless the laws of thermodynamics were violated or other sources of energy were utilized. If cells were able to grow at almost boiling water temperatures, it is more likely that the cells would be at the local ambient temperature. The biophysics and biochemistry of such organisms would then be extremely unusual.

The highest temperatures on the surface of the earth (other than volcanoes) are found in the hot spring areas in Yellowstone National Park and also in Japan, New Zealand, and Iceland. Numerous reports of algae and bacteria found in these springs have been published during the last century. Many different people have claimed that these microorganisms were growing at temperatures as high as 89°C (*1*). In the laboratory, however, the highest growth temperatures which have been confirmed are 72° to 75°C (*2*). These experiments might suggest a maximum temperature for growth, and it is therefore of interest to use a metabolic test for growth of organisms reported in the hot springs.



Although the evidence is scanty, a plausible explanation for a maximum temperature is the limitation of amino acid acceptance by soluble RNA. Whatever its molecular basis, it is clear that there is a maximum temperature for active life processes. The earlier ecological reports which have been widely quoted must therefore be reinterpreted as survival without metabolism. The limiting factors which prevent life forms as we know them from evolving at boiling water temperatures is worthy of further research (*8*).

Kempner 1963 Science

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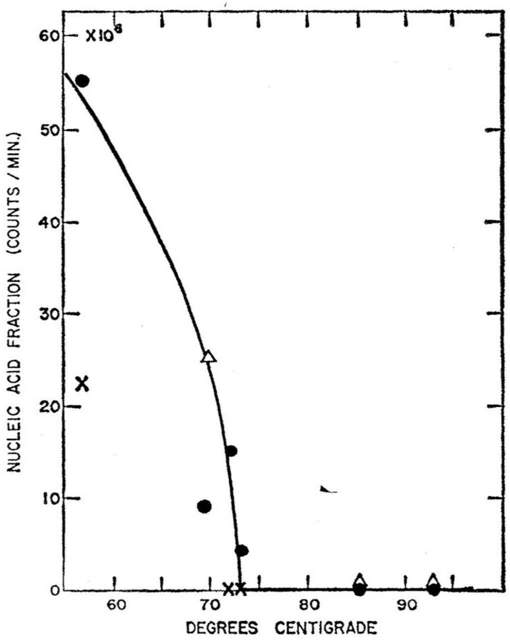

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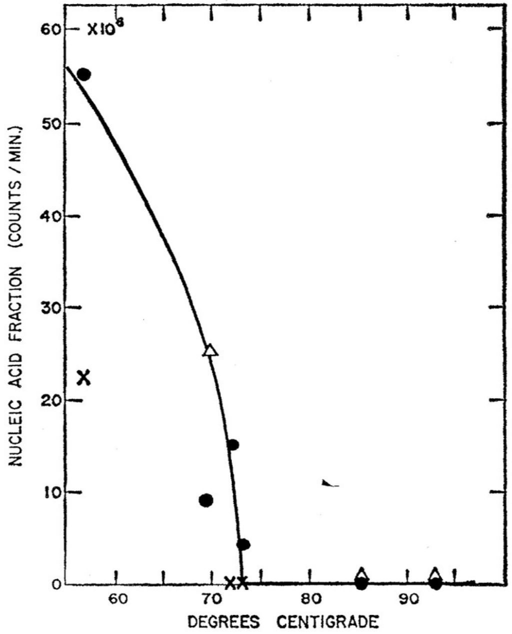

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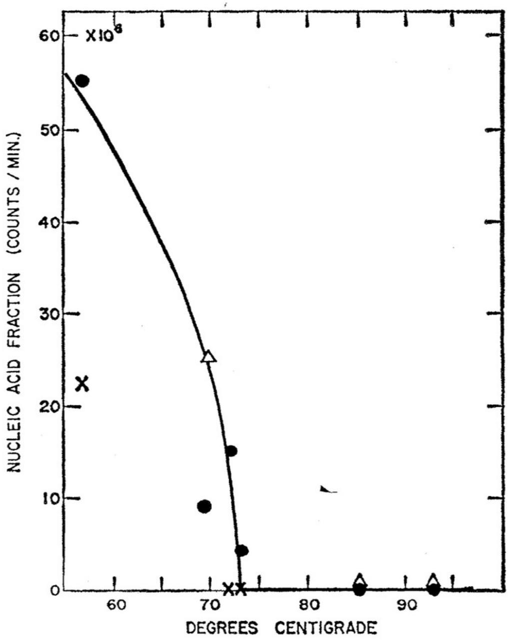
Upper Temperature Limit of Life

Abstract. *Samples of microorganisms from the hot springs of Yellowstone National Park have been collected and tested for the ability to utilize radioactive phosphorus. No evidence for growth was found above 73°C.*

Survival of organisms under extreme conditions may depend on isolation of their internal environment from inordinate salt concentrations, *p*H, or even pressure. However, there would be no defense against high temperatures unless the laws of thermodynamics were violated or other sources of energy were utilized. If cells were able to grow at almost boiling water temperatures, it is more likely that the cells would be at the local ambient temperature. The biophysics and biochemistry of such organisms would then be extremely unusual.

The highest temperatures on the surface of the earth (other than volcanoes) are found in the hot spring areas in Yellowstone National Park and also in Japan, New Zealand, and Iceland. Numerous reports of algae and bacteria found in these springs have been published during the last century. Many different people have claimed that these microorganisms were growing at temperatures as high as 89°C (*1*). In the laboratory, however, the highest growth temperatures which have been confirmed are 72° to 75°C (*2*). These experiments might suggest a maximum temperature for growth, and it is therefore of interest to use a metabolic test for growth of organisms reported in the hot springs.



Although the evidence is scanty, a plausible explanation for a maximum temperature is the limitation of amino acid acceptance by soluble RNA. Whatever its molecular basis, it is clear that there is a maximum temperature for active life processes. The earlier ecological reports which have been widely quoted must therefore be reinterpreted as survival without metabolism. The limiting factors which prevent life forms as we know them from evolving at boiling water temperatures is worthy of further research (*8*).

Kempner 1963 Science

A (very) brief history of thermophiles

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Thermus aquaticus gen. n. and sp. n., a Non-sporulating Extreme Thermophile

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The isolation of a new thermophilic bacterium, *Thermus aquaticus* gen. n. and sp. n., is described. Successful enrichment requires incubation at 70 to 75 C, and the use of nutrient media relatively dilute with respect to the organic components. Strains of *T. aquaticus* have been isolated from a variety of thermal springs in Yellowstone National Park and from a thermal spring in California. The organism has also been isolated from man-made thermal habitats, such as hot tap water, in geographical locations quite distant from thermal springs. Isolates of *T. aquaticus* are gram-negative nonsporulating nonmotile rods which frequently form long filaments at supraoptimal temperatures or in the stationary phase. All isolates form a yellow cellular pigment, probably a carotenoid. A characteristic structure formed by all isolates is a large sphere, considerably larger than a spheroplast. These large spheres, as well as lysozyme-induced spheroplasts, are resistant to osmotic lysis. Deoxyribonucleic acid base compositions of four strains were determined by CsCl density gradient ultracentrifugation and found to be between 65.4 and 67.4 moles per cent guanine plus cytosine. The growth of all isolates tested is inhibited by fairly low concentrations of cycloserine, streptomycin, penicillin, novobiocin, tetracycline, and chloramphenicol. Nutritional studies on one strain showed that it did not require vitamins or amino acids, although growth was considerably faster in enriched than in synthetic medium. Several sugars and organic acids served

In 1965 Brock observed and described pink filamentous bacteria from hot springs in Yellowstone National Park living at temperatures of 82-88 °C.

In 1969 he published the isolation of Thermus aquaticus, an organisms isolated actively growing at temperatures between 65-80 °C.

From then onward the upper limit was considered to be 85 °C.

A (very) brief history of thermophiles

Ultrathin mycelia-forming organisms from submarine volcanic areas having an optimum growth temperature of 105 °C

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The most extremely thermophilic organisms known to date have been isolated from continental volcanic areas[1–3], and grow optimally between 70 and 85 °C. In the hope of finding organisms living at temperatures above 100 °C I have taken samples from the hot sea floor of a submarine solfatara field where, as a result of the high pressures liquid water is found that is hotter than 100 °C. Here I report that, from these samples, I isolated unusual disk-shaped prokaryotic organisms, connected by a

to that recently described for a rod-shaped bacterium from Iceland (ref. 7 and F. Fischer, W. Zillig and K. O. Stetter, in preparation).

The enrichment cultures were purified by serial dilution. During exponential growth (Fig. 1*a*,*b*), the pure cultures exhibited a doubling time of 550 min at 85 °C and of only 220 min at 100 °C (Fig. 1*b*). Surprisingly, the isolates grew optimally at 105 °C with a doubling time of 110 min (Fig. 1*b*). The isolates were transferred (1% inoculation) to fresh medium five times in succession and incubated at 105 °C. Microscopic inspection showed about the same high cell density in the last tube as in the first, indicating that the organisms multiplied at this temperature. At 110 °C, very weak growth occurred (not shown). It remains unclear whether this represents the upper temperature boundary, or whether, more probably, the reduction of growth was due to the sintering of the sulphur forming a lump and therefore rendering it inaccessible to the organisms. Below 80 °C, the isolates were unable to grow (not shown). However, they could be re-isolated from the original anaerobic samples, stored at 4 °C and at −20 °C over a period of at least 10 months. In the presence of oxygen, however, they were inactivated within hours.

Is the CH_4, H_2 and CO venting from submarine hydrothermal systems produced by thermophilic bacteria?

John A. Baross, Marvin D. Lilley & Louis I. Gordon

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Submarine hydrothermal vents are a major source of methane to the oceans[1,2]. The methane, as well as H_2 and CO, are generally believed to result from degassing of the mantle or from abiogenic water–rock reactions[1], a conclusion supported by direct correlations between ^3He and CH_4, and generally between CH_4, H_2 and CO and dissolved silicate in hydrothermal waters[2,3]. An alternative source for these gases might be microbiological. This would imply that active bacterial communities exist in deep-sea hot water environments, some of which have

Submarine hydrothermal systems located along tectonic rifts and ridges off the Galapagos Islands and along the East Pacific Rise[4–6] and the Gorda Ridge have been studied and sampled since 1977. Two groups of vents have been described: (1) cracks and small fissures which emit warm water (5–22 °C) at a flow rate of $\sim 2\ cm\ s^{-1}$ and (2) spectacular 3–10-m high conical sulphide mounds (chimneys) out of which spout super-heated waters with temperatures as high as 380 ± 30 °C and flow rates of metres per second (ref. 5). At the depth of these hydrothermal systems (2,500–2,650 m) seawater can remain liquid at temperatures up to ~460 °C due to the effect of hydrostatic pressure[7]. Sulphide chimneys are found at 21° N along the East Pacific Rise, and the warm water vents are found at both the Galapagos Rift and at 21° N. Associated with each of these hydrothermal environments are extensive and diverse groups of animals which are now known to derive their energy from chemosynthetic bacteria[8,9]. These bacteria, which are found in large numbers in warm vent waters[4,9], on animal and rock surfaces[10,11], and as endosymbionts associated with specific vent animals[12], utilize S, metals and possibly organic gases and H_2 as energy sources. Bacteria had previously not been thought to exist in the super-heated waters associated with sulphide chimneys.

In 1982, Karl Stetter and colleagues isolated and described a new Archaea isolated from shallow water hydrothermal vents from Sicily capable of growing at 105 °C optimum temperatures, the genus Pyrodictium.

At the same time John Baross and colleagues described organisms capable of similar growth temperatures.

This pushed the boundaries of thermophiles above the 100 °C mark.

A (very) brief history of thermophiles

Ultrathin mycelia-forming organisms from submarine volcanic areas having an optimum growth temperature of 105 °C

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(What we believe is) the maximum temperature has been increasing ever since…

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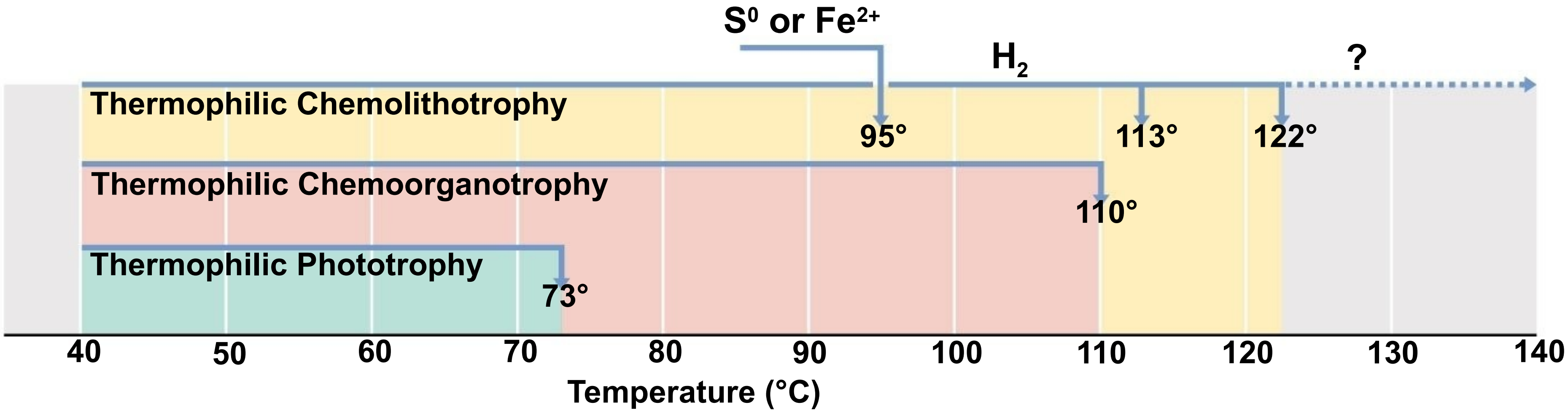
Remember:

The upper temperature limit of life (small letter) depends on many factors and it is metabolism specific…



This week read

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Baross, J. A., Lilley, M. D., and Gordon, L. I. (1982). Is the CH4, H2 and CO venting from submarine hydrothermal systems produced by thermophilic bacteria? Nature 298, 366–368. doi:10.1038/298366a0.