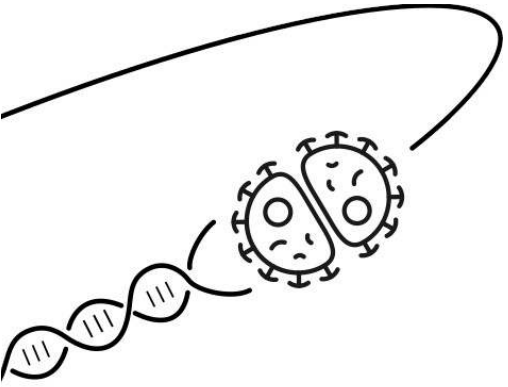
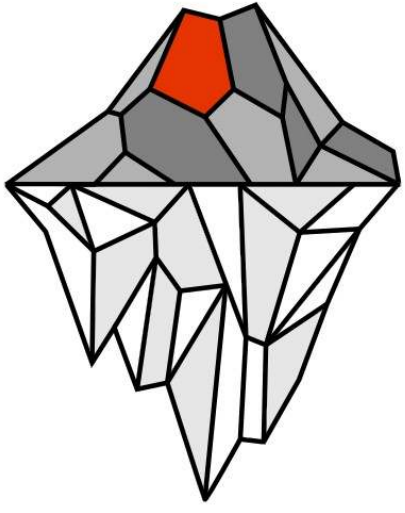


MICROBIOLOGY OF EXTREME ENVIRONMENTS

Pressure as a parameter for bacterial growth



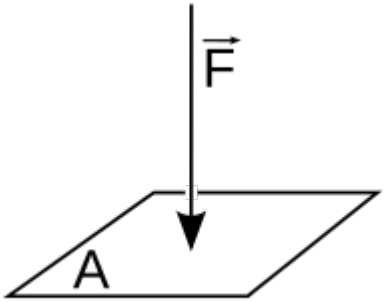
GIOVANNELLI LAB

BIOLOGY OF EXTREME ENVIRONMENTS

DIPARTIMENTO DI BIOLOGIA

Pressure

Pressure is the force applied perpendicular to the surface of an object per unit area over which that force is distributed



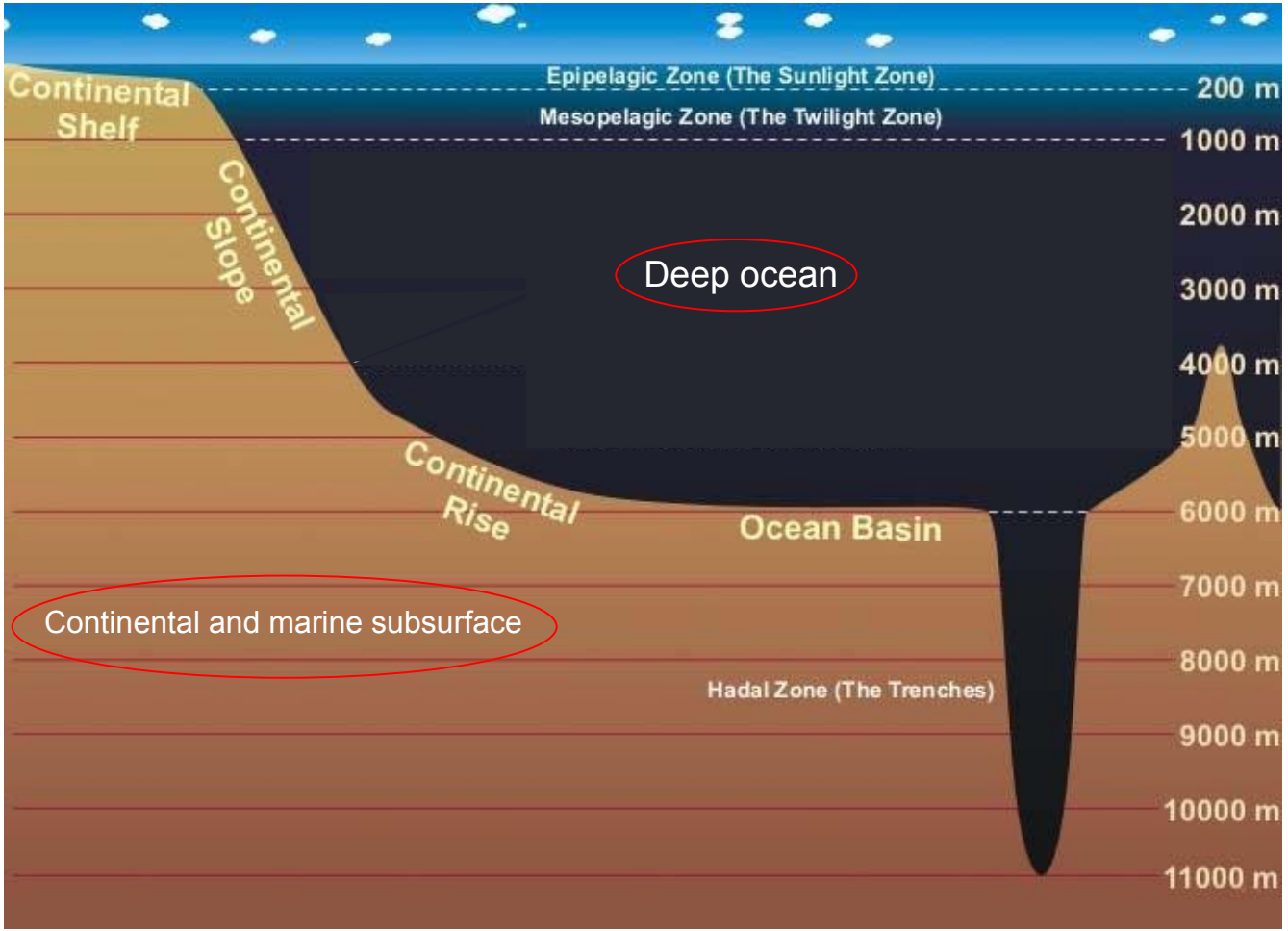
SI unit for pressure is **Pascal**

Atmospheric pressure (1 atm) = 1 bar = 101,325 Pa = **0.1 MPa**

Hydrostatic pressure = 10 MPa/km

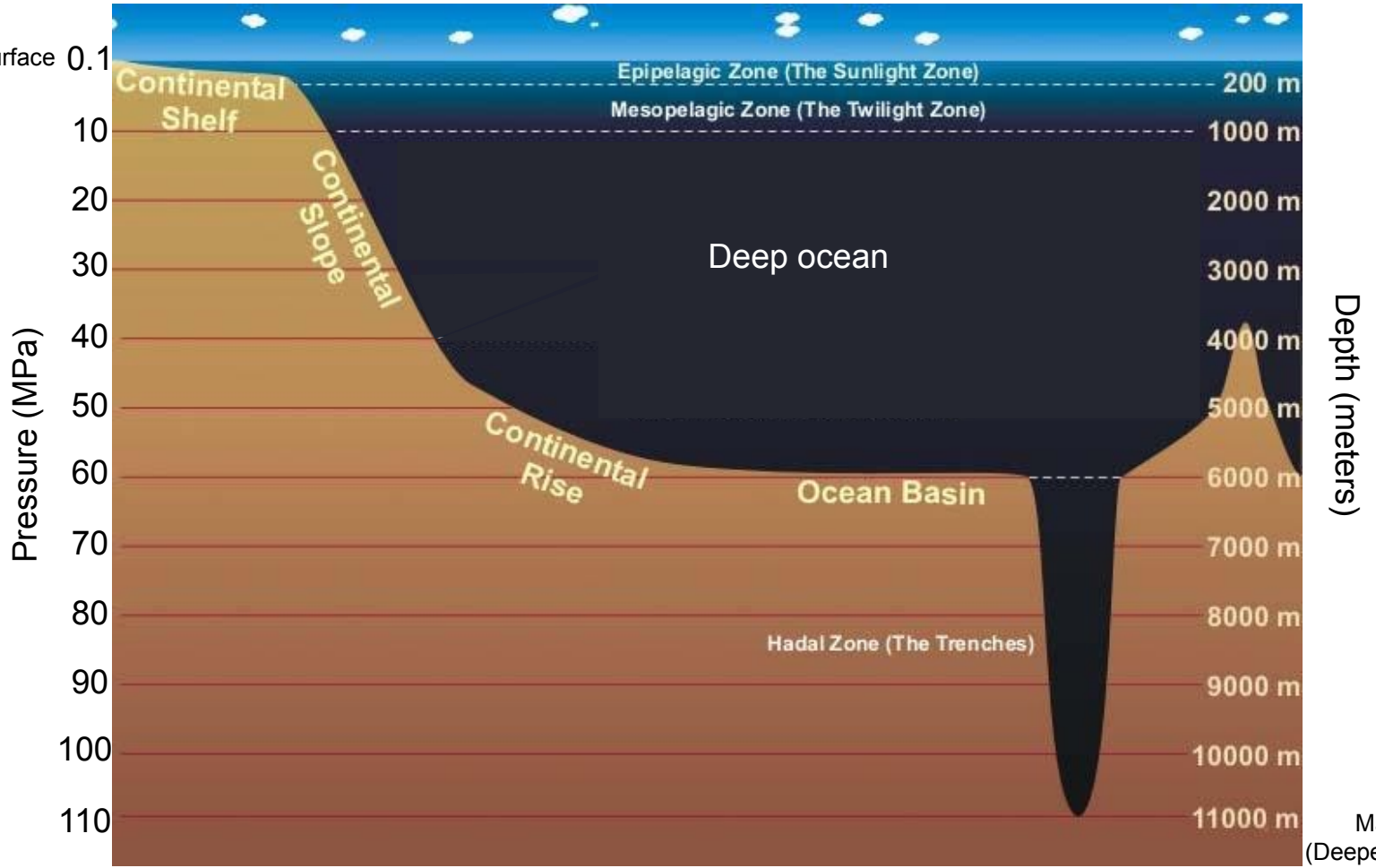
Lithostatic pressure = 25 MPa/km

High pressure environments



https://news.nationalgeographic.com/news/2014/12/141219-deepest-fish-mariana-trench-animal-ocean-science/

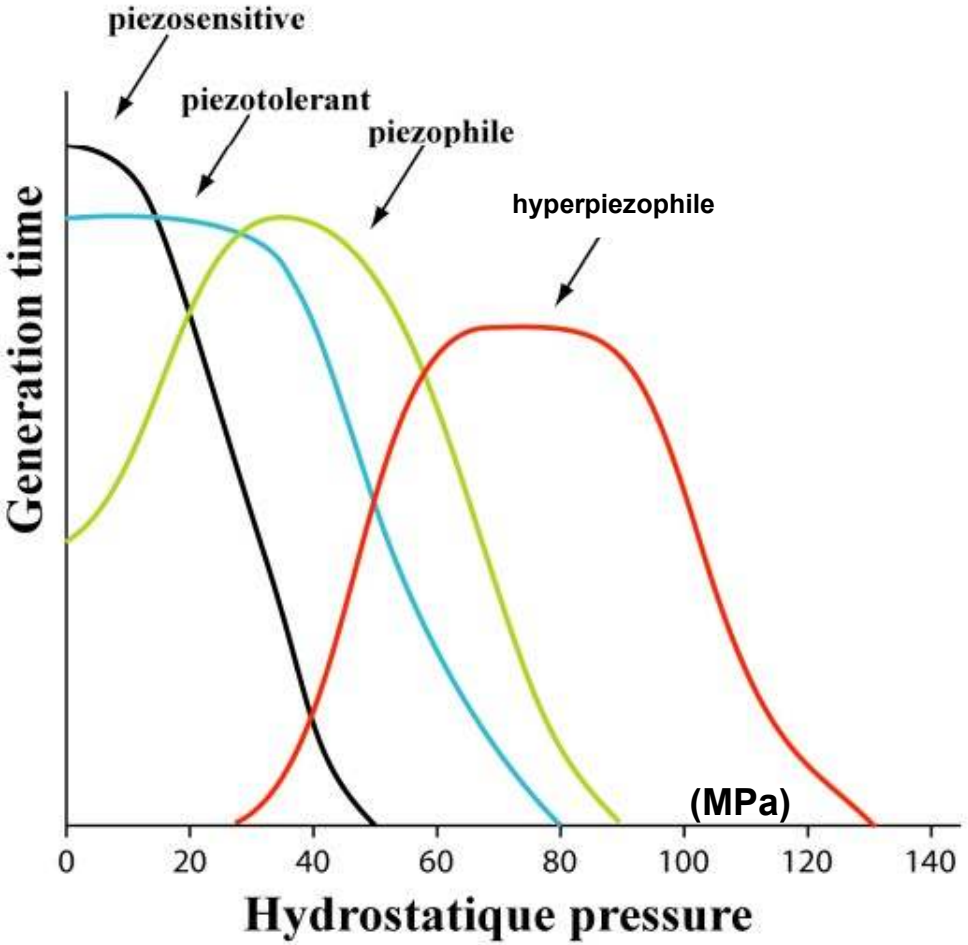
Pressure range on Earth' surface



https://news.nationalgeographic.com/news/2014/12/141219-deepest-fish-mariana-trench-animal-ocean-science/

Pressure effect on microbes growth

Piezo derive from Greek and it means **to press**



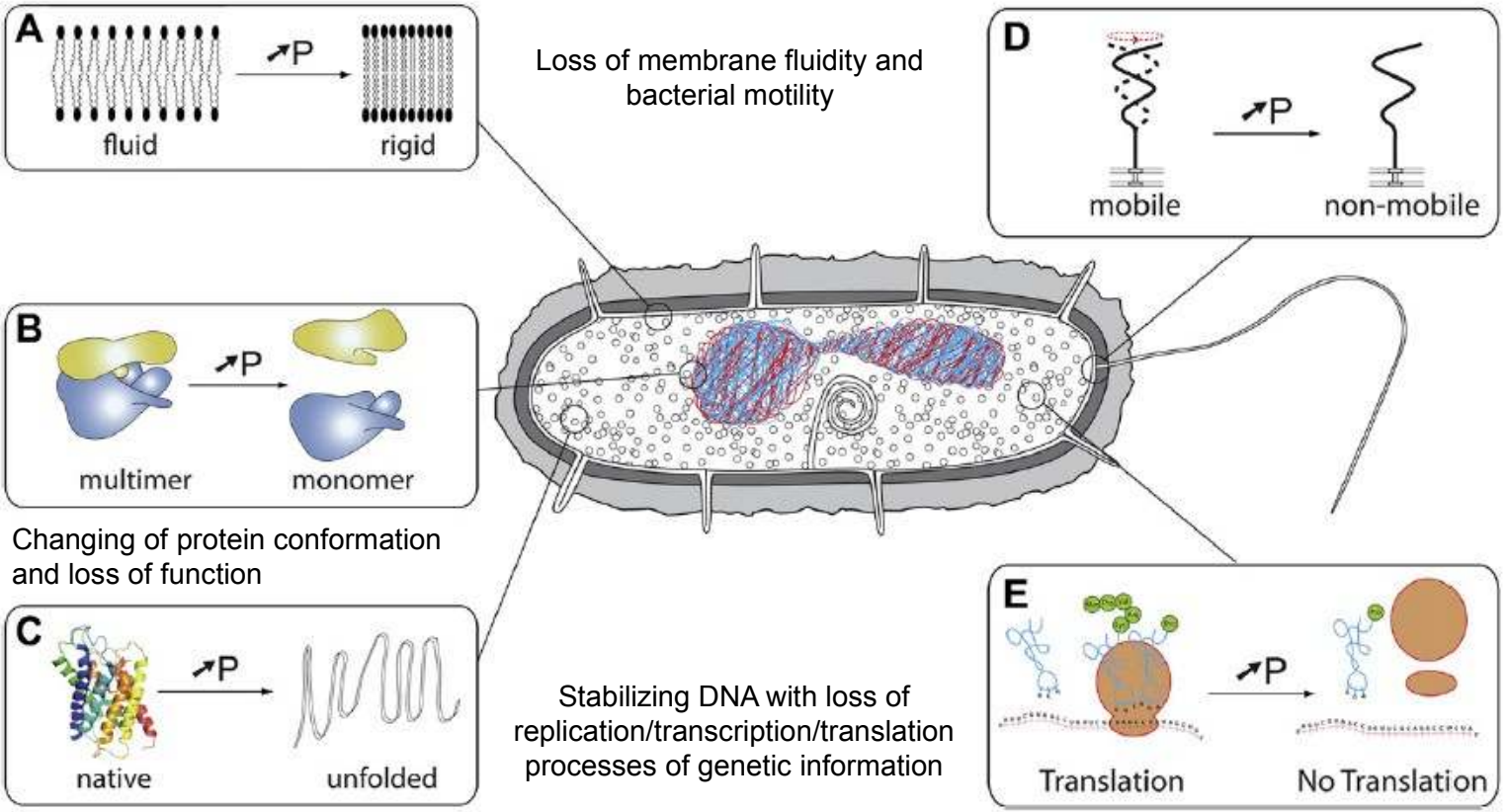
- Piezosensitive → 0.1 MPa
- Piezotolerant → 0.1 - 10 MPa
- Piezophile → 10 - 50 MPa
- Hyperpiezophile → >50 MPa

Castell et al. 2020

Effect of pressure on microbes

Conformational changing of macromolecules with subsequent loss of function

Does not have effect on covalent bonds (e.g. Temperature)



Oger and Jebbar 2010

Effect of pressure on microbes

Table 1
Cellular processes/structures impaired by high hydrostatic pressure in *E. coli*.

Process	Pressure-abolishing process (MPa)	Reference
Motility	10	Meganath and Marquis (1973)
Substrate transport (isopropylthiogalactopyranoside)	26	Landau (1967)
Cell division	20–50	Zobell and Cobet (1962, 1963)
Growth	50	Yayanos and Pollard (1969)
DNA replication	50	Yayanos and Pollard (1969)
Translation	60	Yayanos and Pollard (1969)
Transcription	77	Yayanos and Pollard (1969)
Viability	200	Pagan and Mackey (2000)

Most of what we know about the effect of pressure on bacteria comes from experiment with *Escherichia coli*, a **mesophilic piezotolerant bacterium → DEFINITELY NOT AN EXTREMOPHYLE**

Piezophilic microbes are difficult to isolate and cultivate

Oger and Jebbar 2010

Strategies to cope with extreme pressures

Cell membrane packed with unsaturated fatty acids → Increase membrane fluidity at high pressure

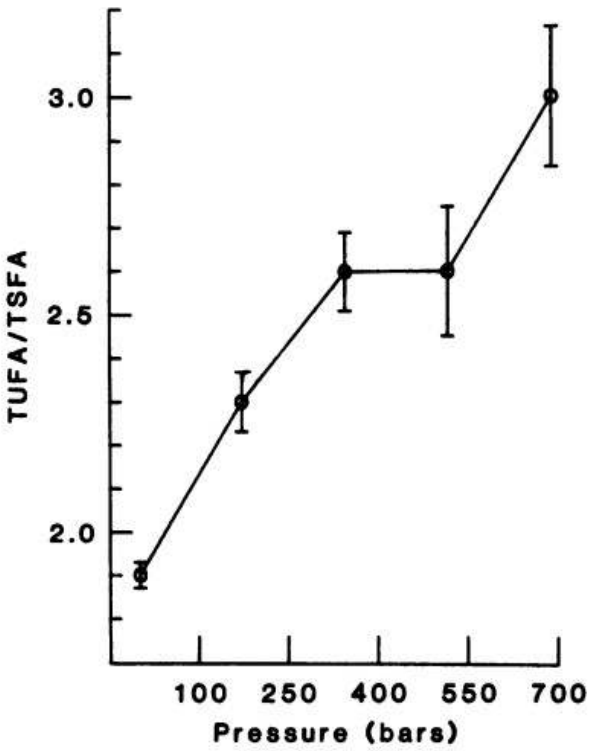
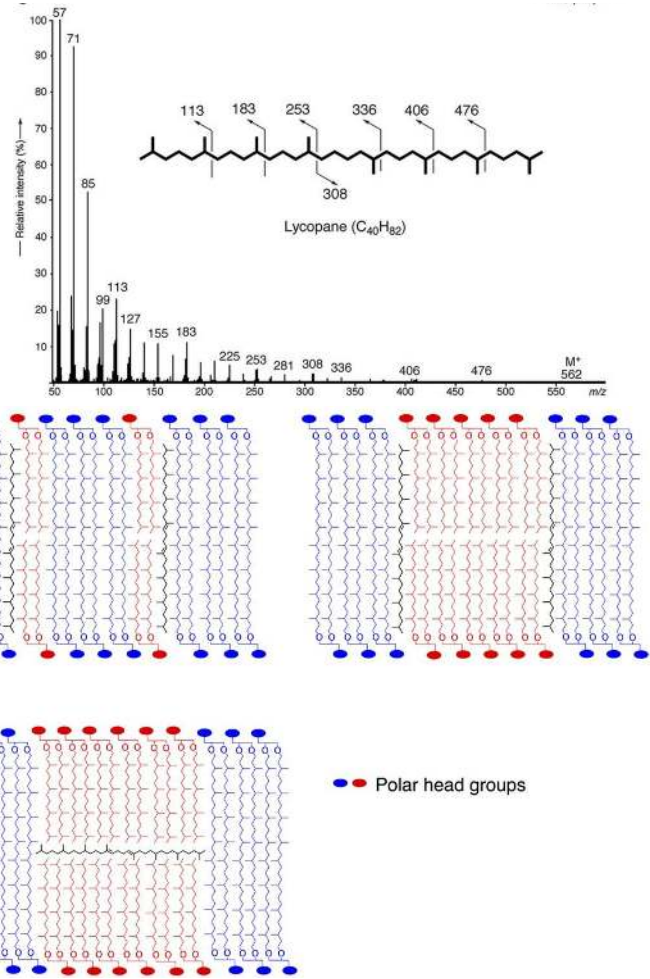


Fig. 1. Ratio of total unsaturated fatty acids (TUFA) to total saturated fatty acids (TSFA) as a function of pressure. Data points are means ± standard errors for five separate experiments.

DeLong & Yayanos 1985



Cairo et al. 2015

Strategies to cope with extreme pressures

Intracellular salt content and osmolyte regulation

At high pressure and high salt content, bacteria increase the intracellular concentration of osmolytes

β-hydroxybutirrate (**β**-HB)

High concentration of osmolyte → integrity of the cell

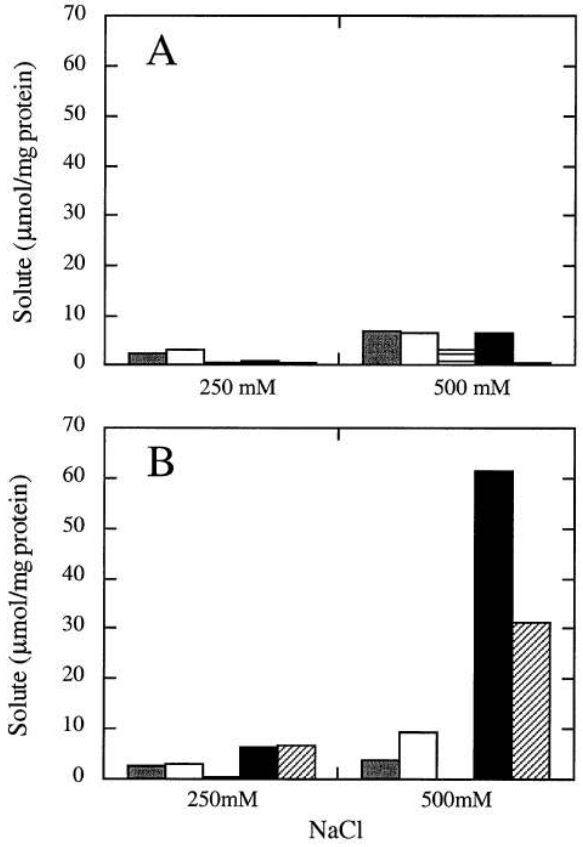


Fig. 5. Distribution of intracellular solutes in *P. profundum* cells grown at **A** 0.1 MPa or **B** 28 MPa to early stationary phase as a function as external NaCl: ▩, betaine; □, glutamate; ⊟, alanine; ■, β-HB; ▨, β-HB oligomer

Martin et al. 2002

Strategies to cope with extreme pressures

Specific high-pressure gene expression

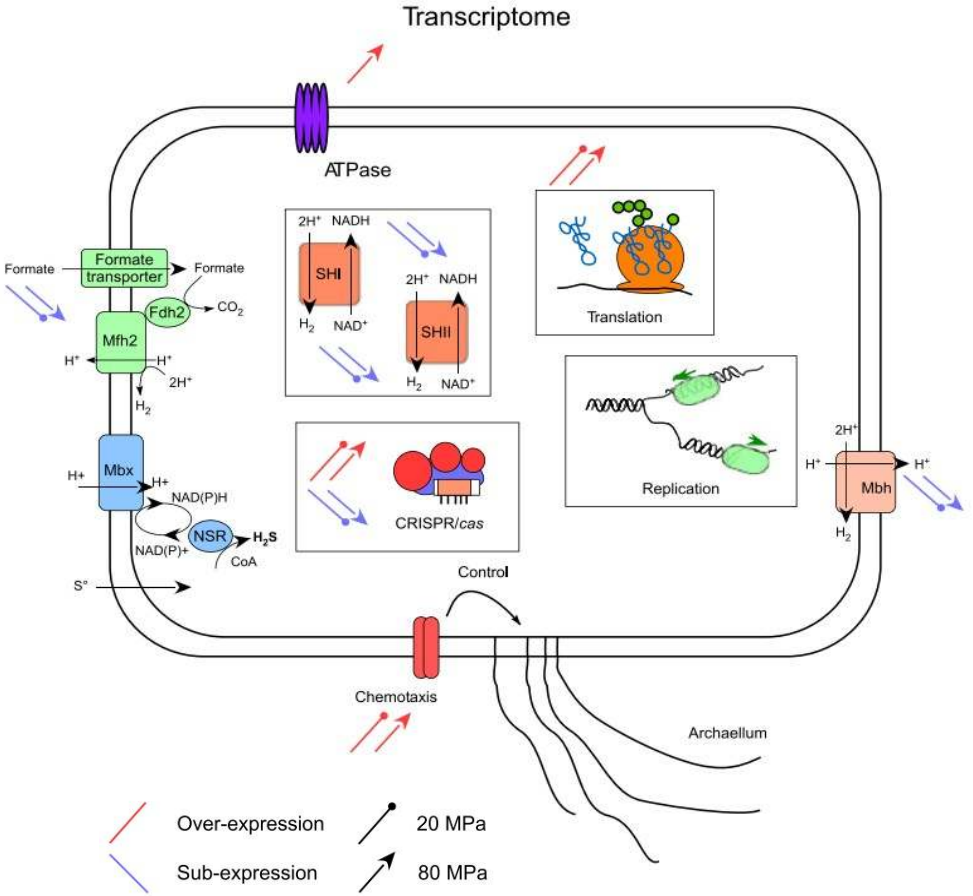
Under high pressure:

↓ Mfh2 hydrogenases → repression of H2 metabolism

↑ Mbx sulfur-dependant hydrogenases → alternative energy supply

↑ chemotaxis machinery → due to change in membrane fluidity

↑ Translation genes → due to disruptive effect of pressure on ribosomes

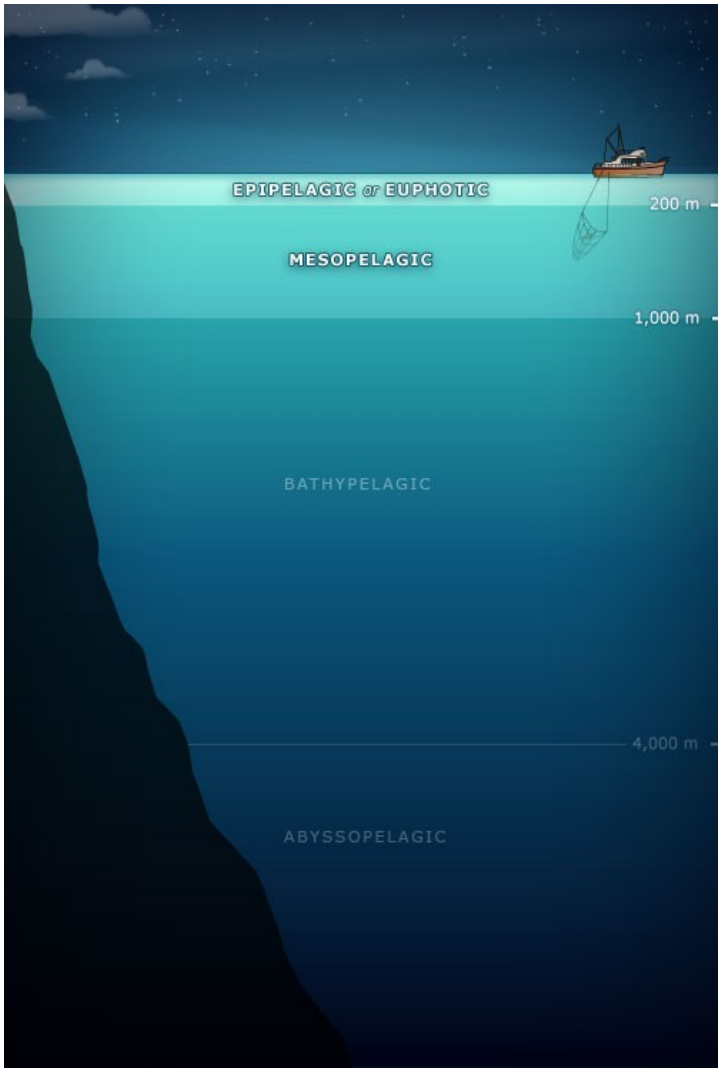


Michaud & Jebbar 2016

WHERE DO PIEZOPHILIC MICROORGANISMS LIVE?

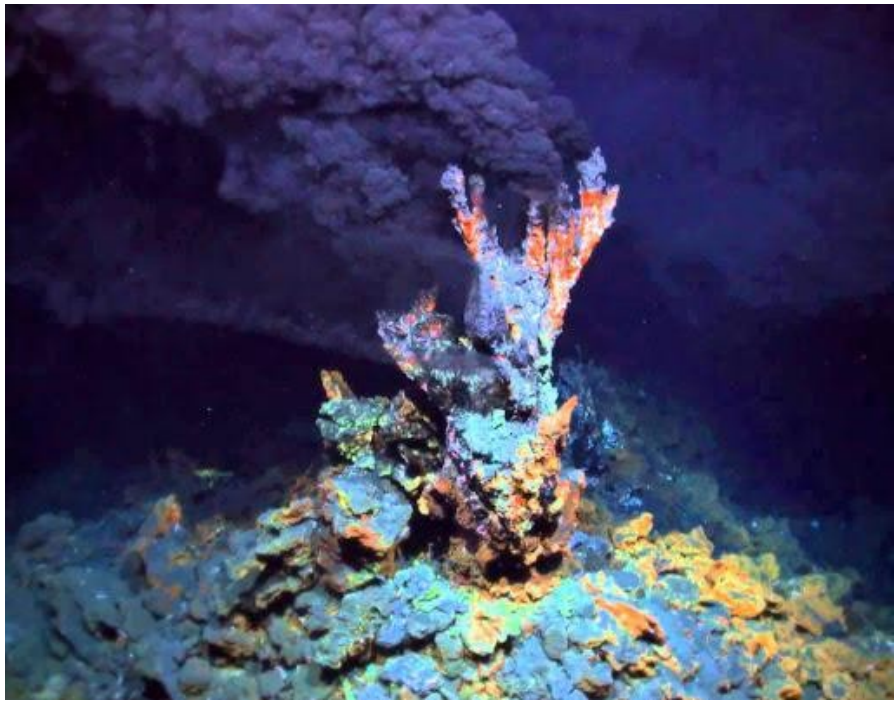
Piezophiles in cold deep ocean

- Deep-sea covers 65% of Earth's surface and holds 88% of total biomass
- Only 5% percent of the oceans have been explored and the 0.01% has been sampled
- Average depth of the ocean → 3,800 meters
- Average pressure in the ocean → 38 MPa
- Average deep ocean temperature → 2-4 °C
- No sunlight → No photosynthesis → Chemolitoautotrophy
- Oligotrophic water = poor in nutrients
- Psychrophilic piezophilic Bacteria and Archaea



Piezophiles in deep-sea hydrothermal vents

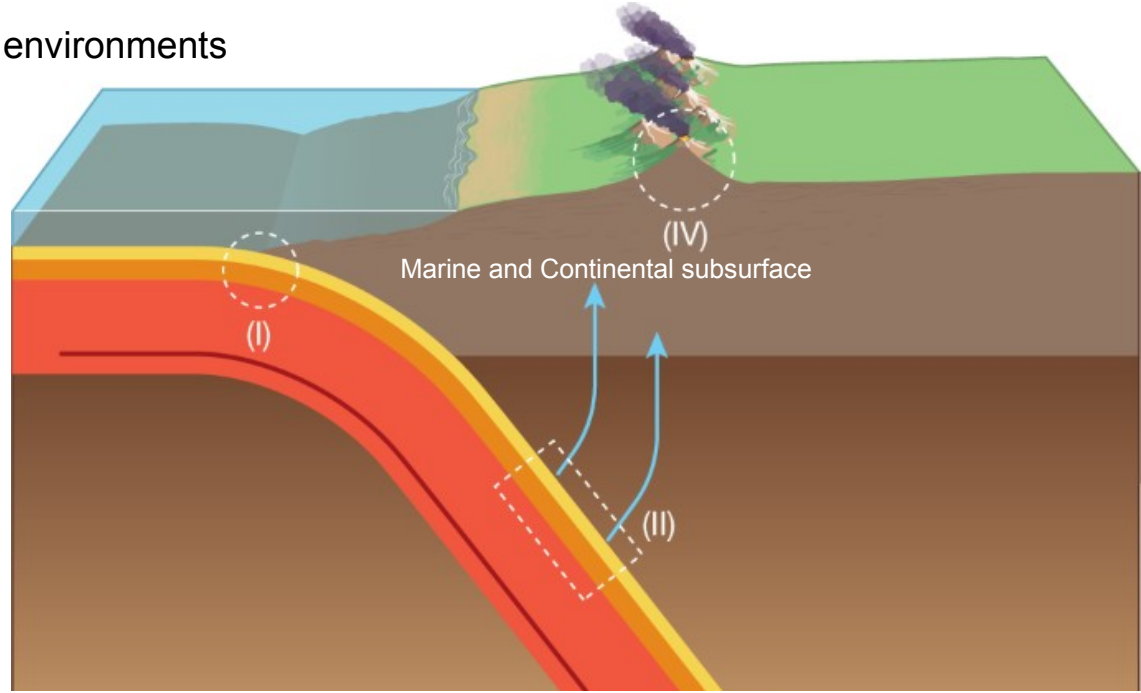
- Environmental fluctuations (e.g. Temperature, salinity, pH)
- Temperatures range between 460 and 2 °C in just a few cm
- Salinity ranges from 0.1 % to 8 %
- No sunlight → No photosynthesis → Chemolitoautotrophy
- Hyperthermophilic piezophilic Archaea and Bacteria



Jebbar et al. 2015

Subsurface environments

- It is estimated that the subsurface holds about the 15% of the total Earth's biomass
- Here, pressures >110 MPa have been recorded
- Highest pressure = 900 MPa at the top of a subducting plate, Mariana Forearc
- Resident microbial communities play important roles on mediating biogeochemical cycles
- However, it is one of the most unexplored environments



Fullerton et al. 2020

Are we forgetting something?

Low-pressure environments

- Mount Everest → highest peak on Earth with the lowest pressure on the planet (0.0033 MPa)
- Low pressure seems to not have detrimental effect on microbes


https://www.britannica.com/place/Mount-Everest

- Space → lowest pressure. It ranges between 10^{-13} and 10^{-10} MPa (space vacuum)
- Long exposure to space vacuum cause dehydration of DNA


https://it.wikipedia.org/wiki/Stazione_Spaziale_Internazionale

Merino et al. 2019

WHO ARE THEY?

Piezophilic isolates

To date, only about 56 facultative and obligate piezophile, both Archaea and Bacteria, are known

Archaea

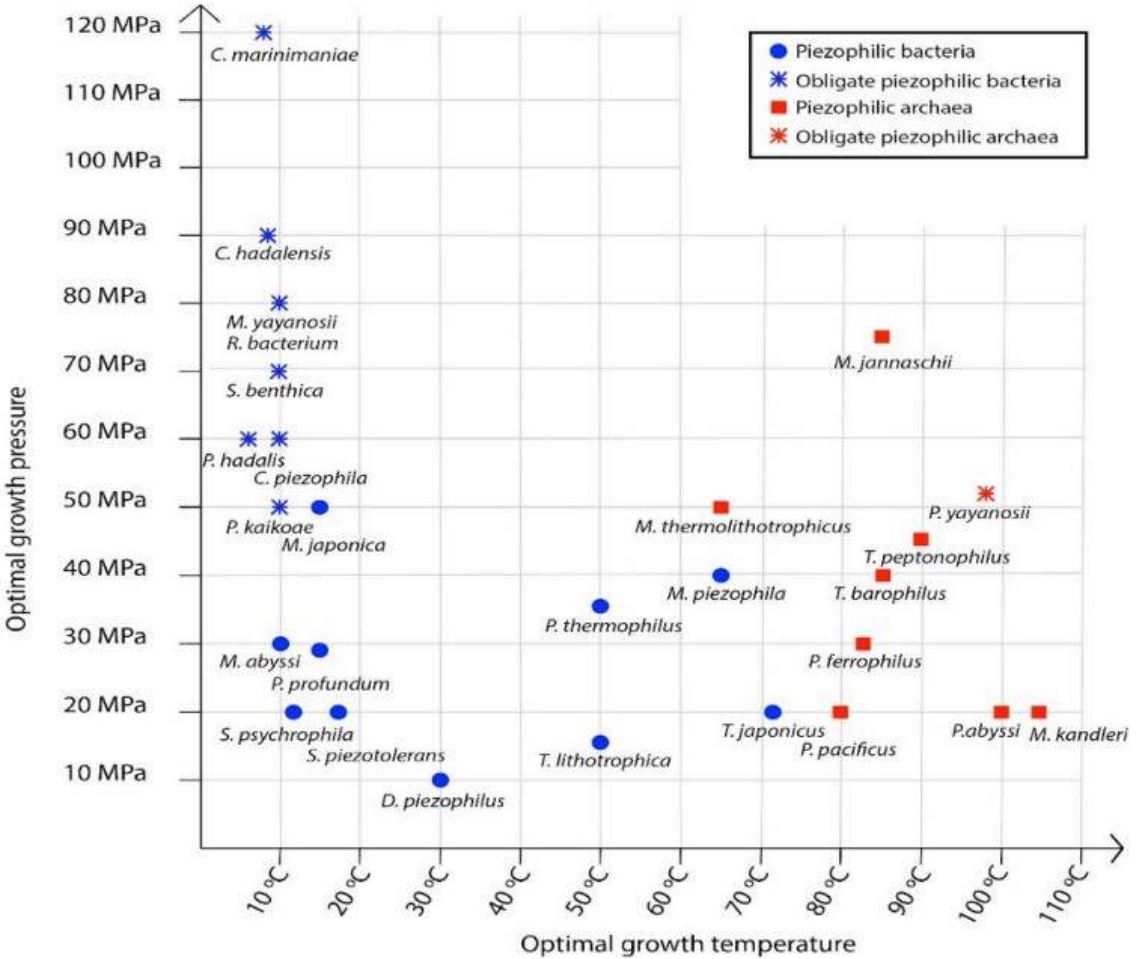
- Euryarchaeota
- Crenarchaeota
- Asgardarchaeota

Bacteria

- Gammaproteobacteria (Colwellia, Moritella, Photobacterium and Shewanella)
- Deltaproteobacteria (Desulfovibrio)
- Epsilonbacteria (Marinitoga)
- Bacilli (Carnobacterium)

Lauro & Bartlett 2008, Cario et al. 2019

Pressure Vs Temperature



Castell et al. 2020

Pressure Vs Temperature

Polyextremophile

Table 1. Classification scheme of piezophiles based on optimal growth temperature and pressure[a]

P_{kmax}\T_{kmax}	< 15 °C	15–45 °C	45–80 °C	>80 °C
Piezotolerant (< 10 MPa)	Psychro-piezotolerant	Meso-piezotolerant	Thermo-piezotolerant	Hyperthermo-piezotolerant
Piezophilic (10–50 MPa)	Psychro-piezophile	Meso-piezophile	Thermo-piezophile	Hyperthermo-piezophile
Hyperpiezophilic (> 50 MPa)	Psychro-hyperpiezophile	Meso-hyperpiezophile	Thermo-hyperpiezophile	Hyperthermo-hyperpiezophile

Fang, Zhang & Bazylinski 2010

Colwellia sp., first obligate piezophile psychrophilic bacterium

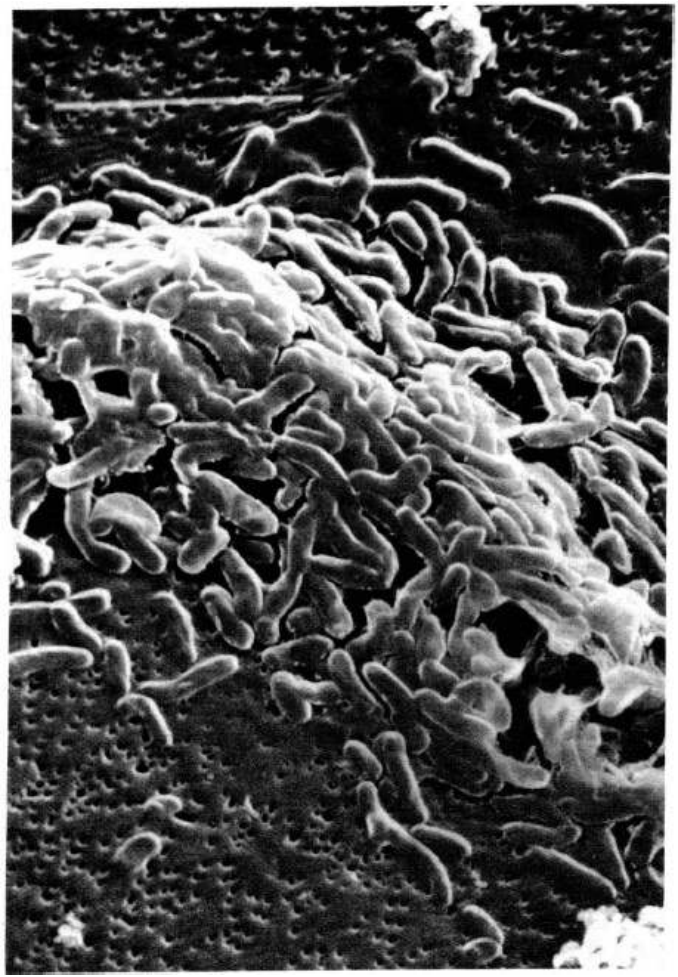
Gram negative bacterium

Strain MT-41

Isolated from a decaying amphipod found at the bottom of the Mariana trench (11,000 meters depth)

Optimal growth pressure = 70 MPa

Optimal growth temperature = 2 °C



Yayanos et al. 1981

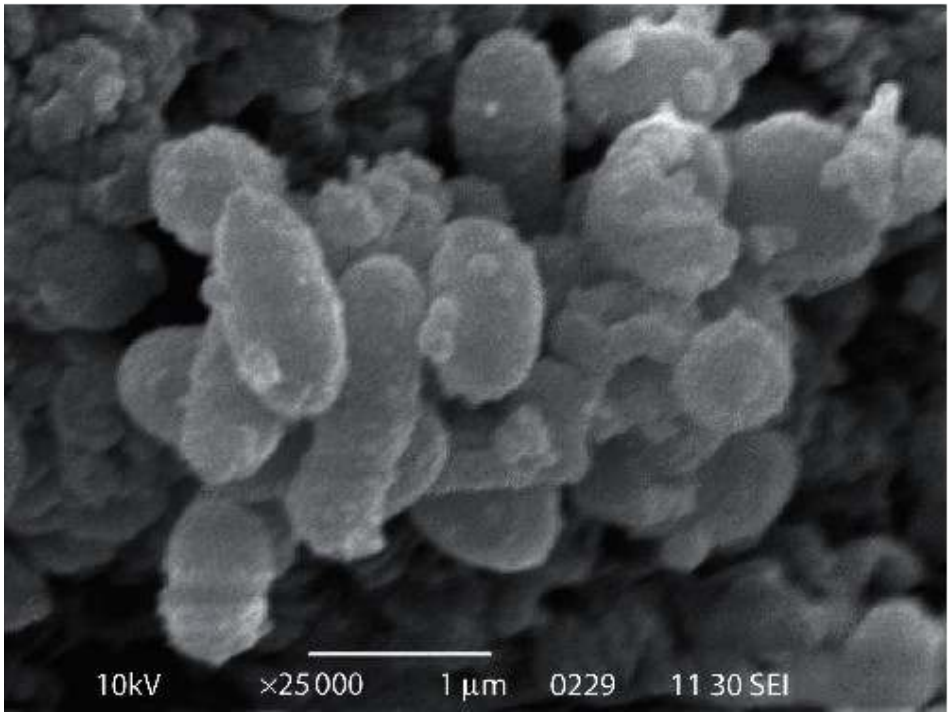
Colwellia marinimaniae, obligate piezophile bacterium with a record

Gram negative bacterium

Rods shape

Optimal growth pressure = 120 MPa

Highest growth pressure = 140 MPa



Kusube et al. 2017

Pyrococcus yayanosii - first obligate piezophilic hyperthermophilic archaeon

Archaeon

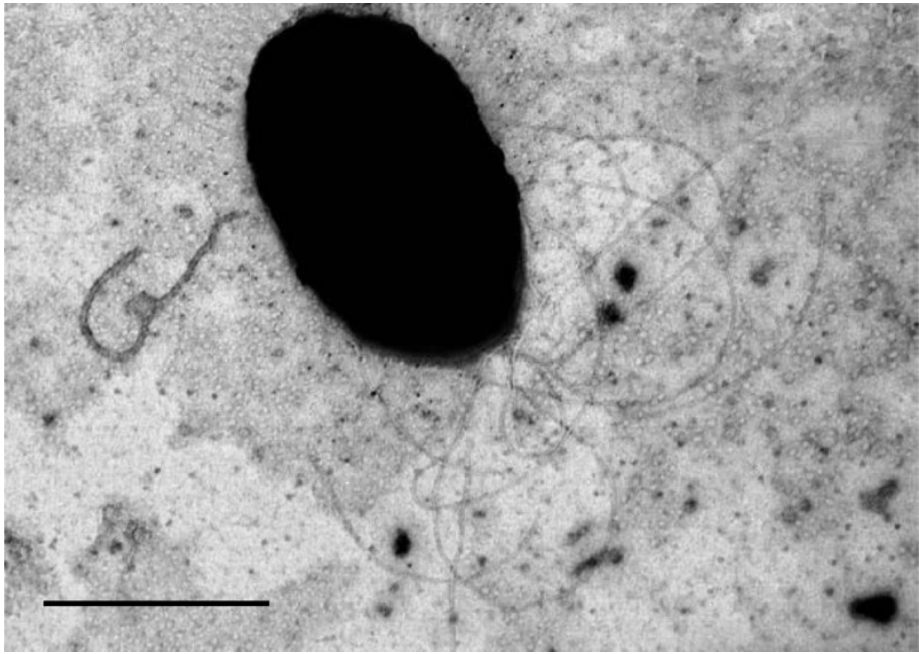
Strain CH1

Isolated from Ashadze site (Mid-atlantic ridge), the deepest hydrothermal vent field explored so far (4 km depth)

Optimal growth pressure = 52 MPa

Optimal growth temperature = 98 °C

Highest pressure growth = 120 MPa

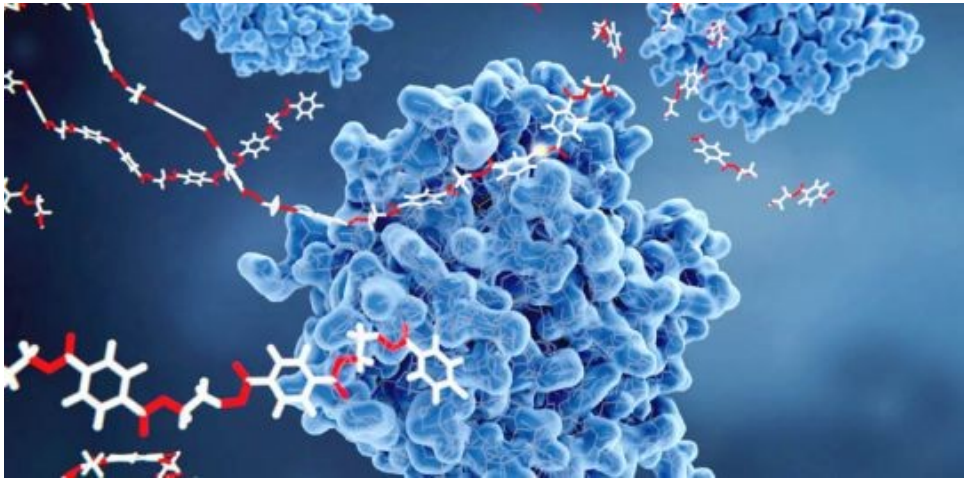


Zeng et al. 2009

Biotech applications and advancements

Food industry

Extremozymes



Biotech applications and advancements

Deep-sea exploration

Pressure-retaining samplers

Jannasch et al. 1976

Suggested readings

- Lauro and Bartlett (2008) - Prokaryotic lifestyles in deep sea habitats
- Oger and Jebbar (2010) - The many ways to cope with pressure
- Jebbar et al. 2005 - Microbial diversity and adaptation to high hydrostatic pressure in deep-sea hydrothermal vents prokaryotes
- Cario et al 2019 - Exploring the Deep Marine Biosphere: Challenges, Innovations, and Opportunities