MICROBIOLOGY OF EXTREME ENVIRONMENTS

Pressure as a parameter for bacterial growth, Xerophiles and radiation resistant extremophiles

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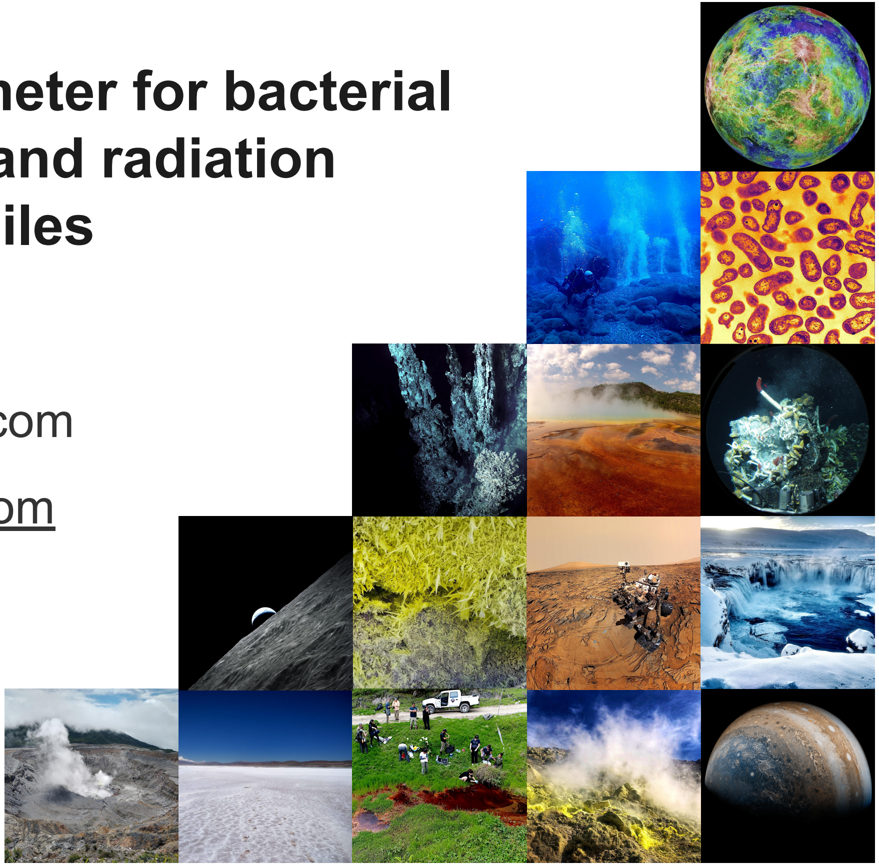


GIOVANNELLI LAB

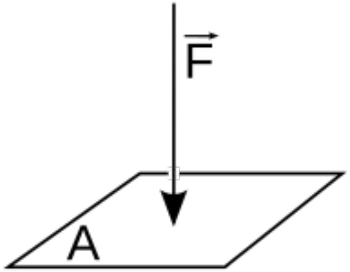
BIOLOGY OF EXTREME ENVIRONMENTS

DIPARTIMENTO DI BIOLOGIA

Class attendance form for contact tracing

Pressure

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



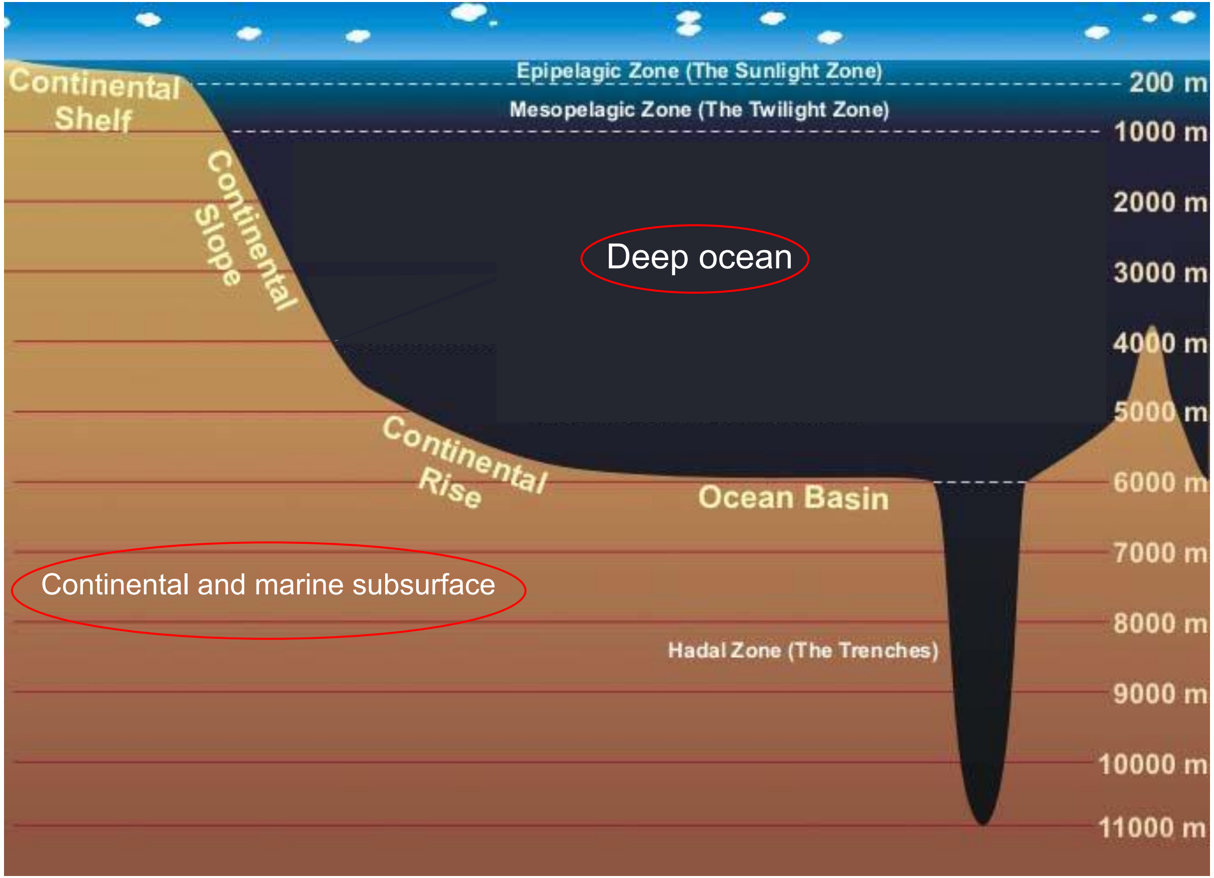
SI unit for pressure is **Pascal**

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

Hydrostatic pressure = 10 MPa/km

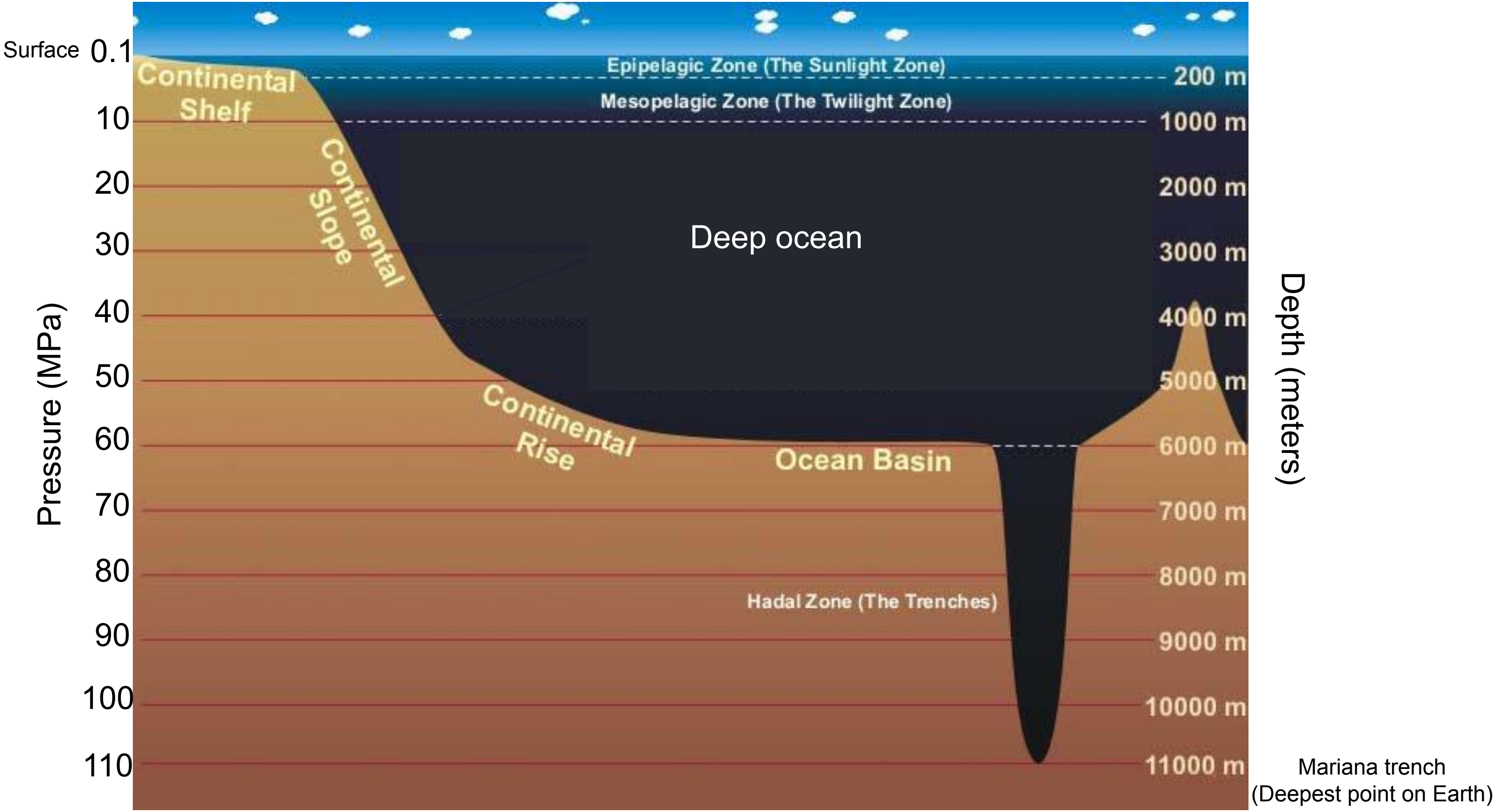
Lithostatic pressure = 25 MPa/km

High pressure environments



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

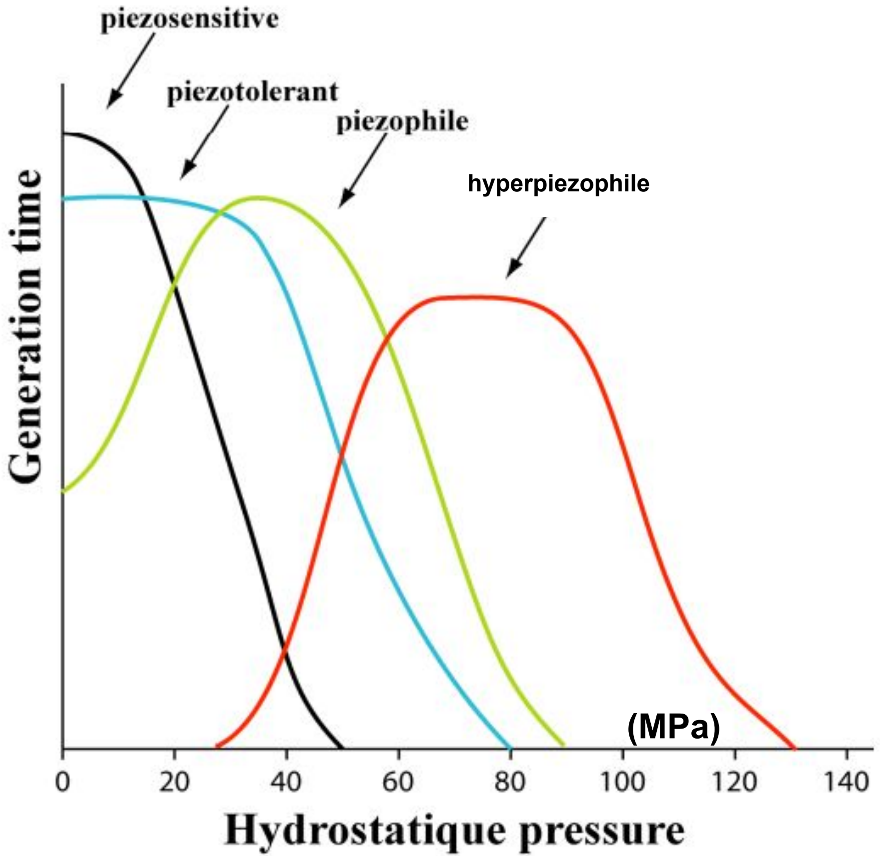
Pressure range on Earth' surface



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**



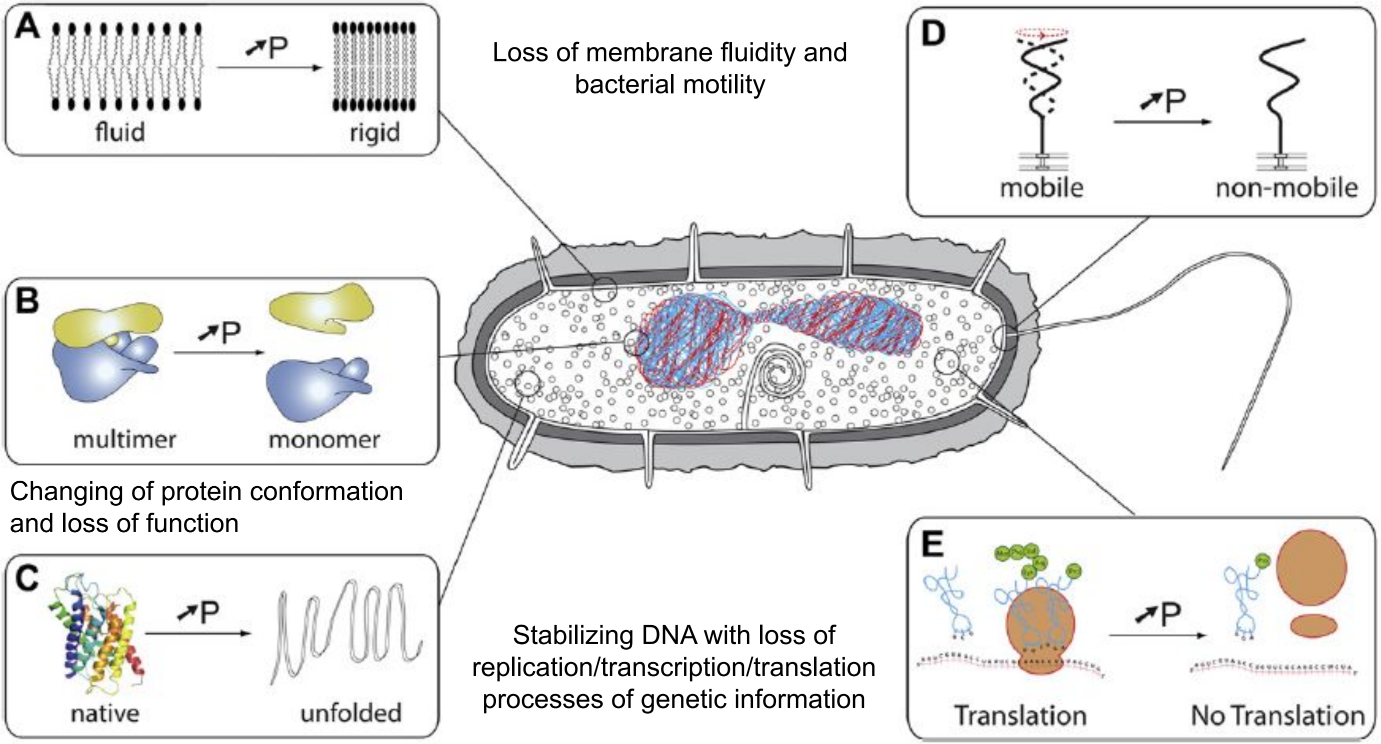
- Piezosensitive → 0.1 MPa
- Piezotolerant → 0.1 - 10 MPa
- Piezophile → 10 - 50 MPa
- Hyperpiezophile → >50 MPa

Castell et al. 2020

Effect of pressure on microbes

<u>Conformational changing of macromolecules with subsequent loss of function</u>

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



Loss of membrane fluidity and bacterial motility

Changing of protein conformation and loss of function

Stabilizing DNA with loss of replication/transcription/translation processes of genetic information

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

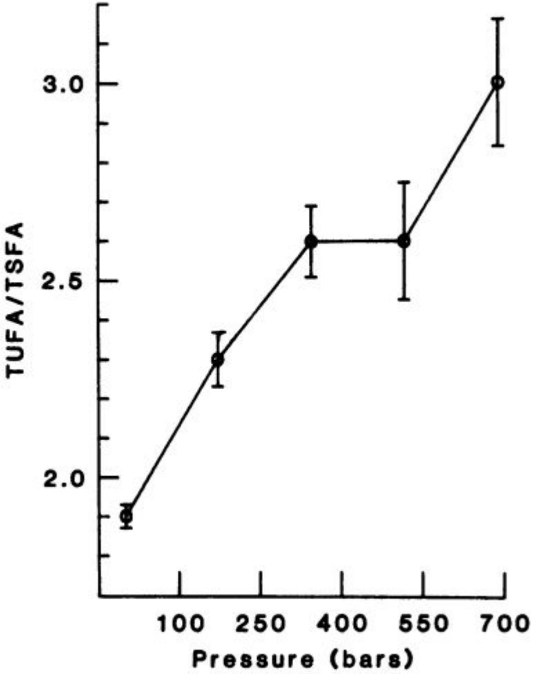
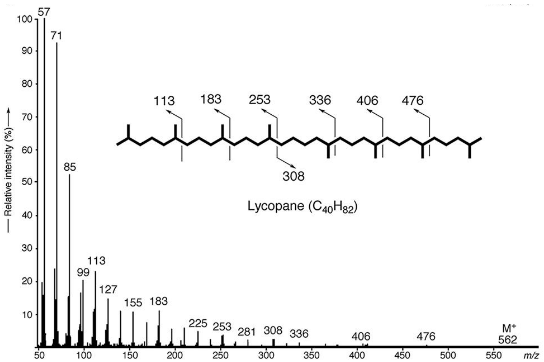
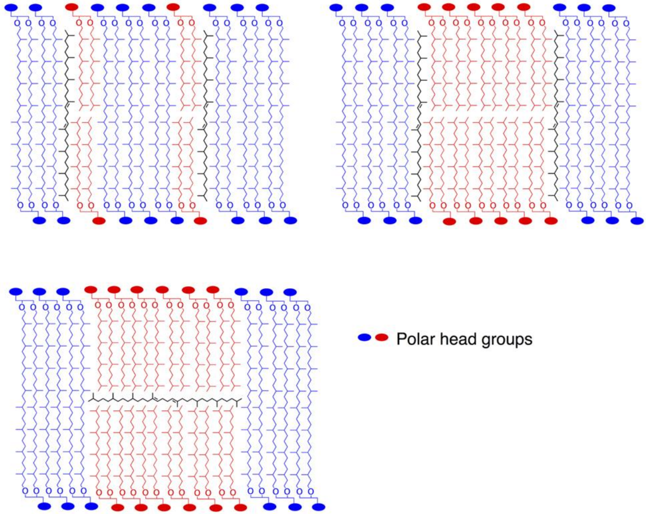


Fig. 1. Ratio of total unsaturated fatty acids (TUFA) to total saturated fatty acids (TSFA) as a function of pressure. Data points are means ± standard errors for five separate experiments.

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

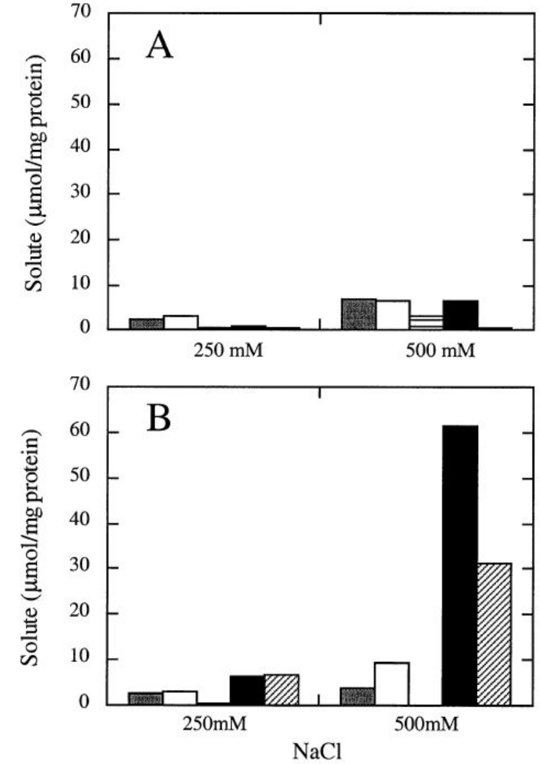


Fig. 5. Distribution of intracellular solutes in *P. profundum* cells grown at **A** 0.1 MPa or **B** 28 MPa to early stationary phase as a function as external NaCl: ▩, betaine; □, glutamate; ⊟, alanine; ■, β-HB; ▨, β-HB oligomer

Martin et al. 2002

Strategies to cope with extreme pressures

Specific high-pressure gene expression

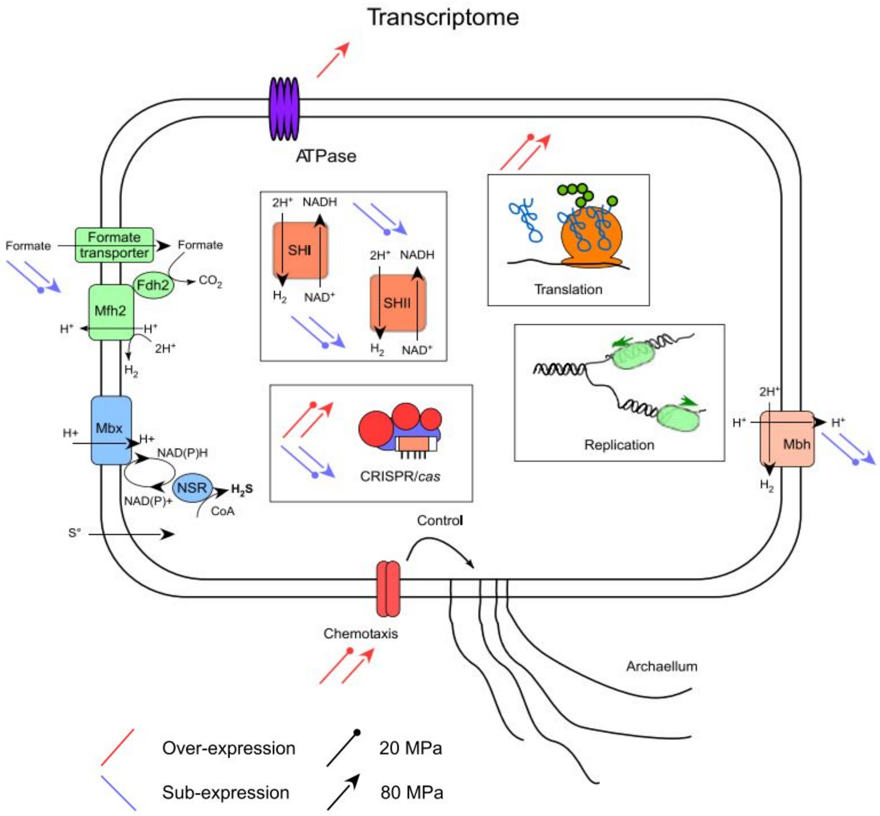
Under high pressure:

↓ 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

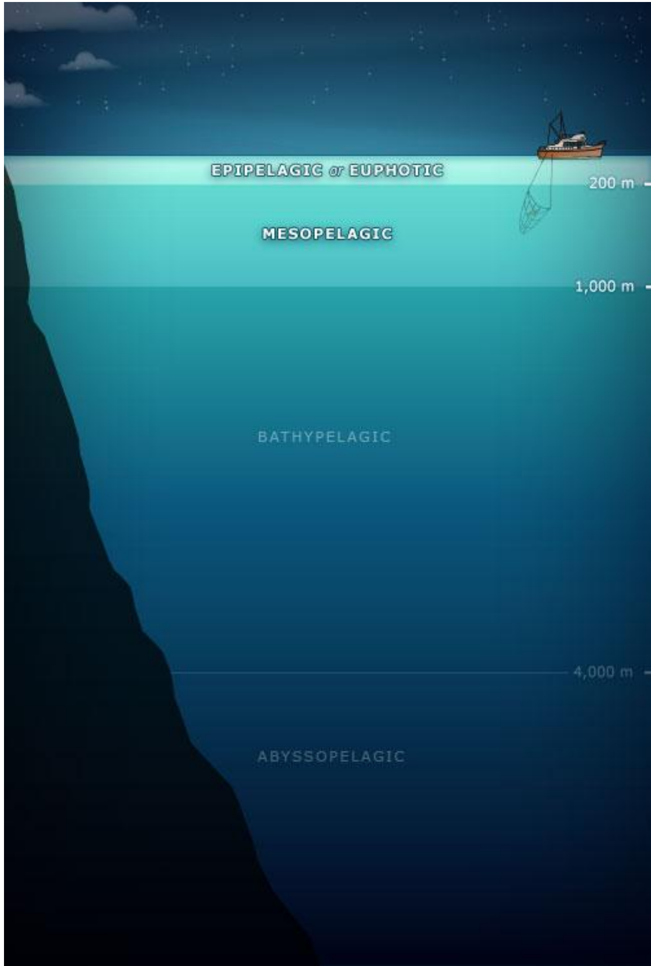


Michaud & Jebbar 2016

WHERE DO PIEZOPHILIC MICROORGANISMS LIVE?

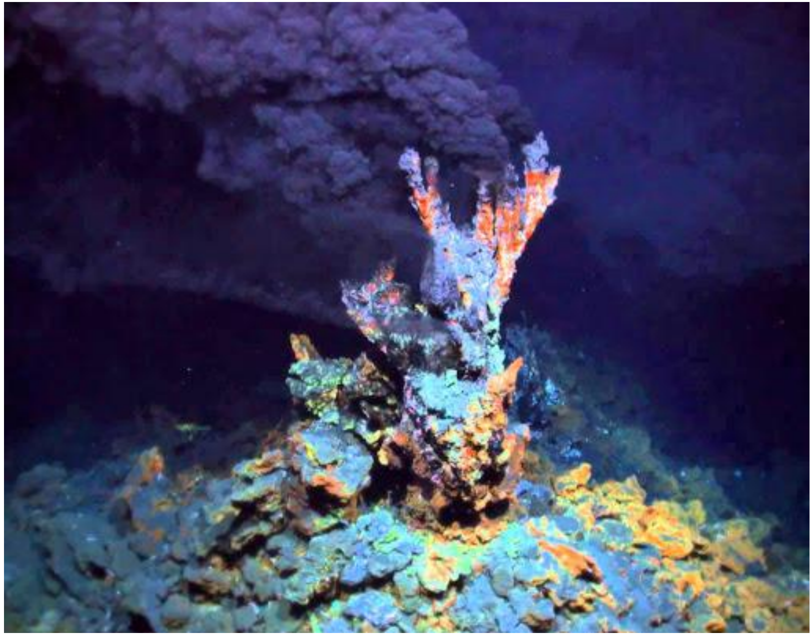
Piezophiles in cold deep ocean

- 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

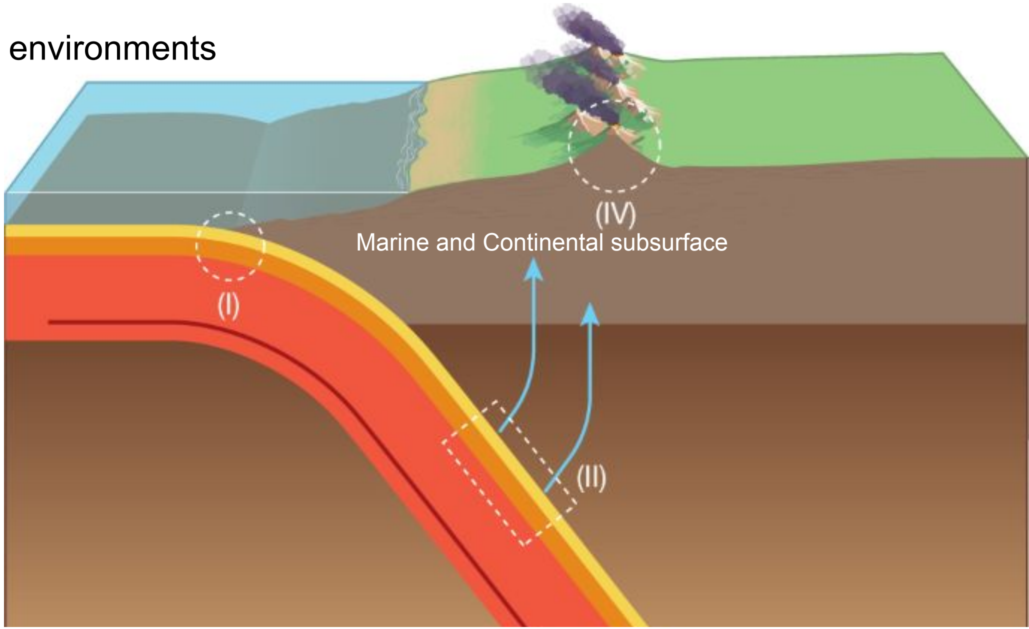
- Environmental fluctuations (e.g. Temperature, salinity, pH)
- Temperatures range between 460 and 2 °C in just a few cm
- Salinity ranges from 0.1 % to 8 %
- No sunlight → No photosynthesis → Chemolitoautotrophy
- Hyperthermophilic piezophilic Archaea and Bacteria



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

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

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

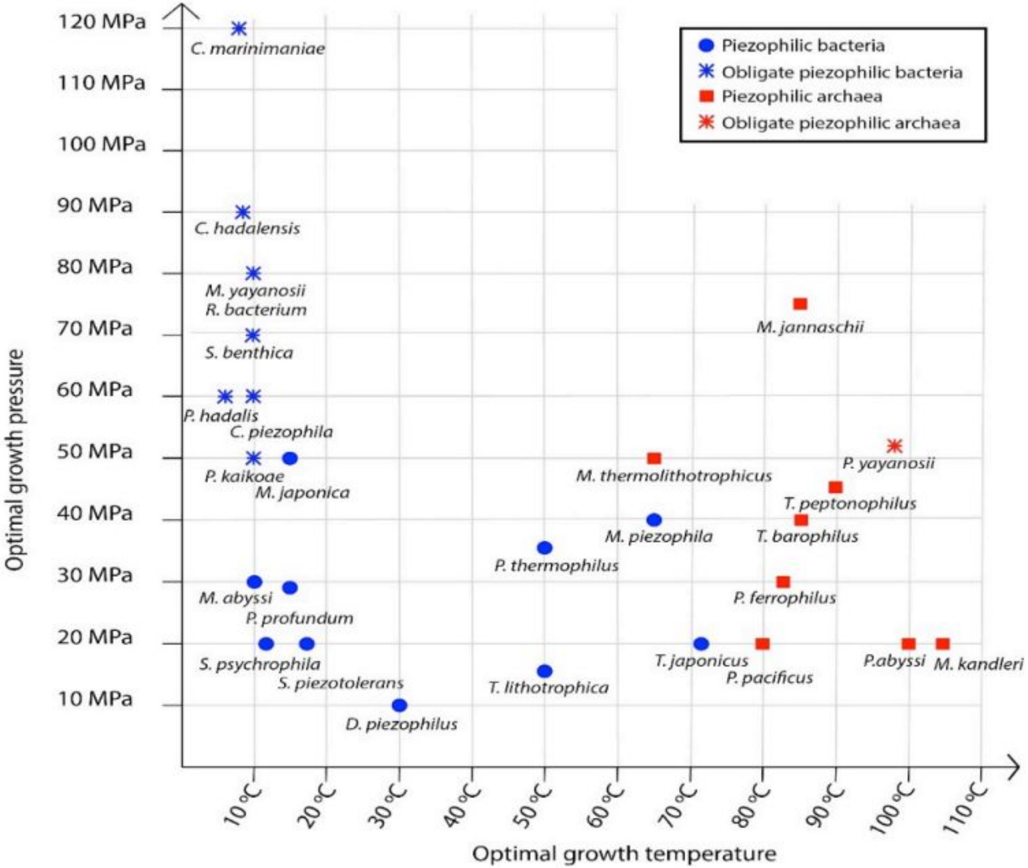
- Euryarchaeota
- Crenarchaeota
- Asgardarchaeota

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]

$P_{kmax} \backslash T_{kmax}$	< 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., <u>first</u> obligate piezophile psychrophilic bacterium

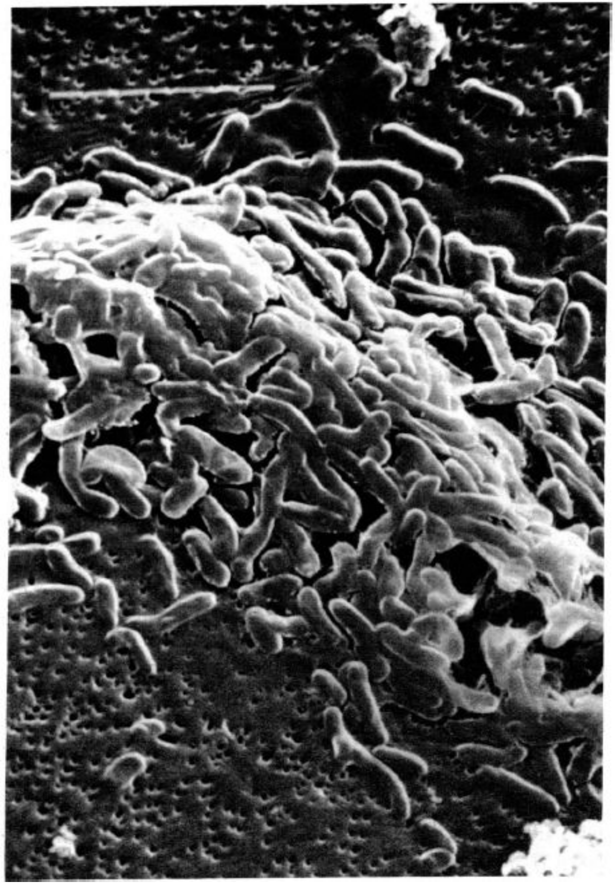
Gram negative bacterium

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

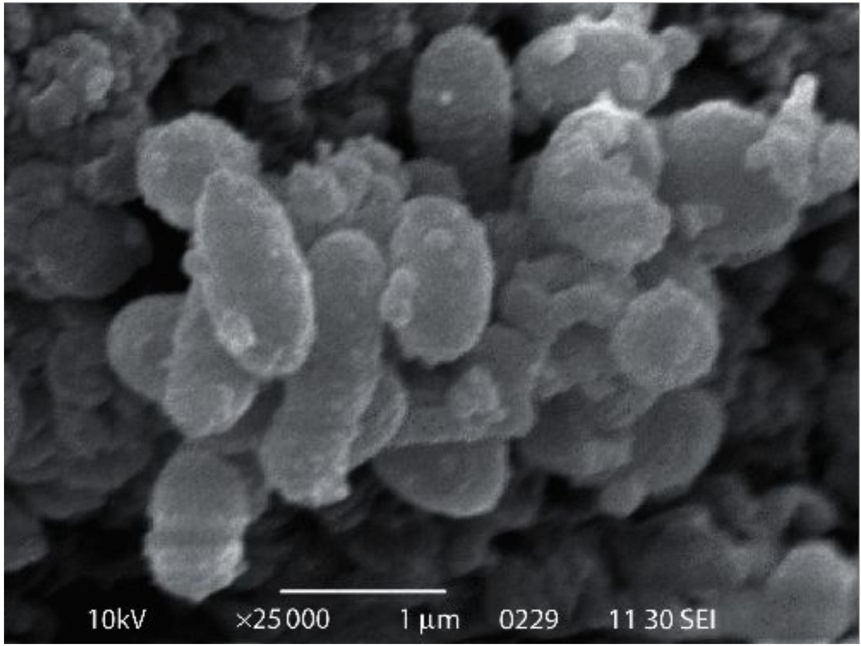
Colwellia marinimaniae, obligate piezophile bacterium with a record

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 <u>archaeon</u>

Archaeon

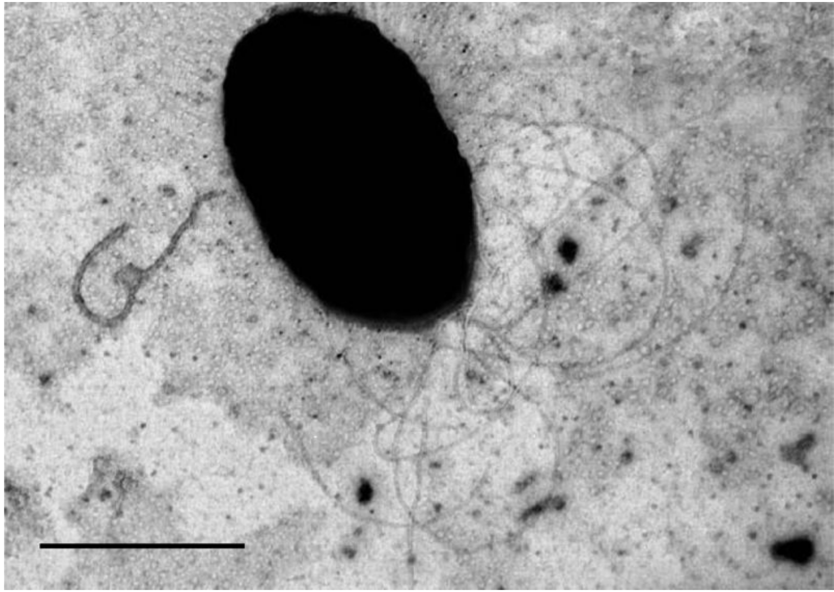
Strain CH1

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

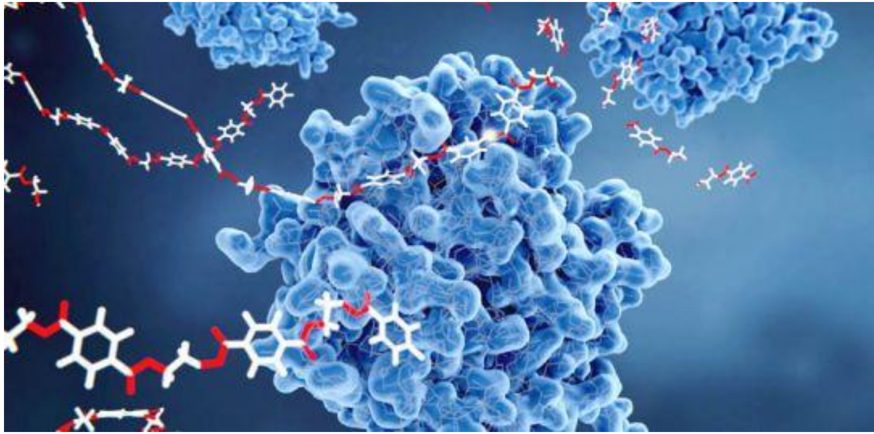


Zeng et al. 2009

Biotech applications and advancements

Food industry

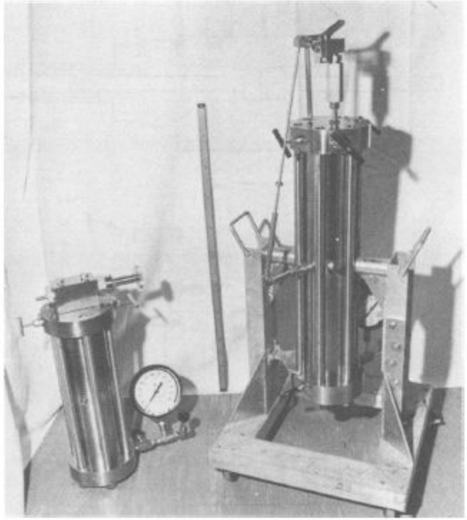
Extremozymes



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

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

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- Archaea

Xerophiles

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- Archaea
- Bacteria

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

- Archaea
- Bacteria
- Fungi

Xerophiles

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Xerophiles

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

~~Xerophiles~~

Xerotolerant

Xeric stress limits

A xeric stress can be caused either by:

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- **Desiccation**, lack of water

Xeric stress limits

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- **Hypertonicity**, excessive solute concentrations in the surrounding environment that removes water from cells

Xeric stress limits

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How do we measure the amount of water available in an environment?

Xeric stress limits

Water activity

Xeric stress limits

Water activity

The vapor pressure of water in a system divided by that of pure water at the same temperature

Xeric stress limits

Water activity

The vapor pressure of water in a system divided by that of pure water at the same temperature

It’s a measure of how much of that water is free and unbound, and thus available for microorganisms to use for growth

Xeric stress limits

Species	Lowest water activity (a_w) for cell division	Environmental source	Refs
Bacteria and archaea			
Haloarchaea GN-2	0.635	Solar salterns, Mexico	3
Haloarchaea GN-5	0.635	Solar salterns, Mexico	3
Halorhabdus utahensis DSM 12940	0.647	Salt Lake, USA	3
Halobacterium strain 004.1	0.658	Brine pool, UK	3
Halorhodospira halophila DSM 244	0.66	Salt lake, USA	3
Salinibacter ruber DSM13855	0.725	Solar salterns, Spain	3
Salisaeta longa DSM 21114	0.747	Dead Sea, Israel	3
Fungi			
Aspergillus penicillioides	0.585	Raisins, Australia	7,106
Xeromyces bisporus	0.637	Antique wood, Thailand	3,6,7,107
Aspergillus amstelodami FRR2792	0.656	Dates, Australia	7
Xerochrysium xerophilum FRR 0530 (formerly known as *Chrysosporium xerophilum*)	0.686	High-moisture prunes, Australia	7
Aspergillus chevalieri PIL 119	0.71	Soiled prunes, Australia	7

Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). *Nature Reviews Microbiology*, *15*(5), 285-296.

Where?

Where?


A
1
2


B
Hyper-arid deserts


Hyper-arid deserts


hypersaline aquatic environments

Where?


Salt-cured food

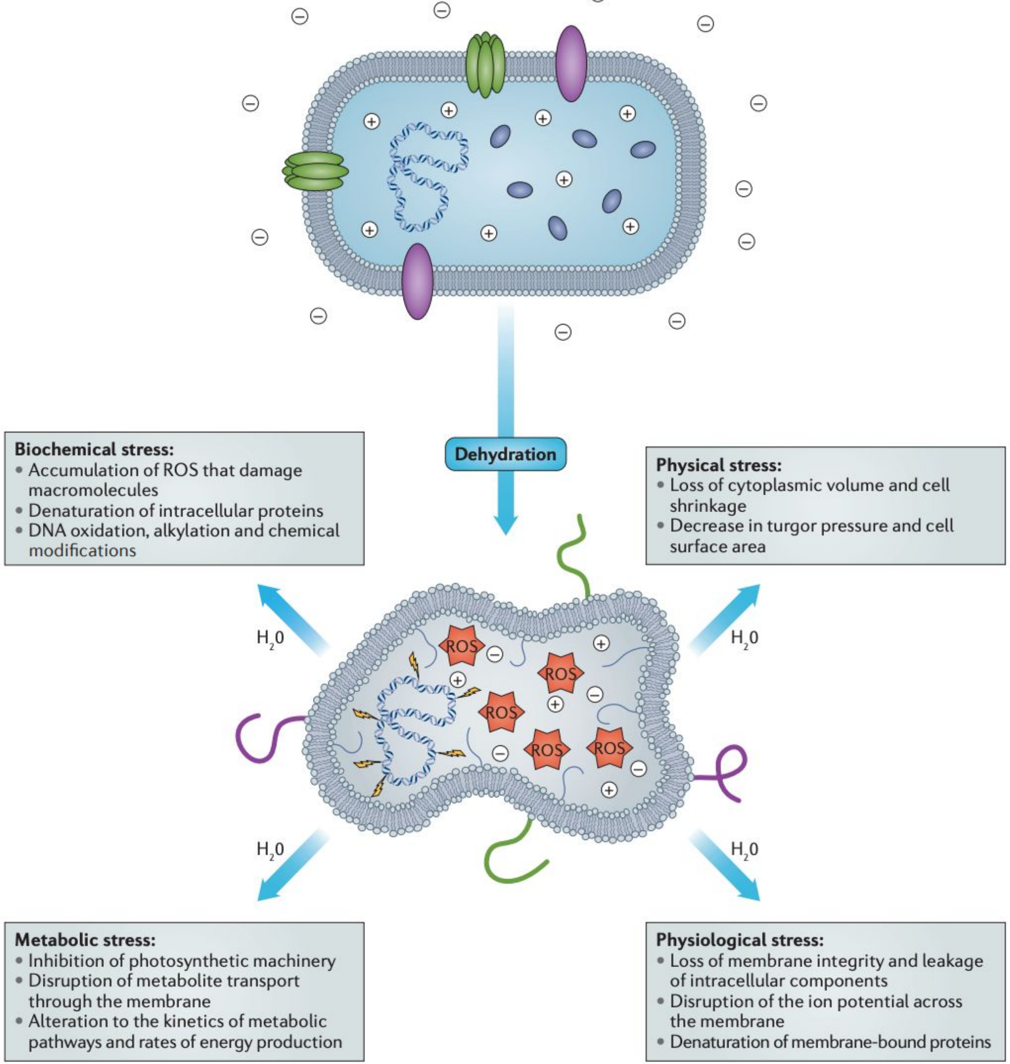

A
1
2


B
Hyper-arid deserts


Hyper-arid deserts


hypersaline aquatic environments

What happens when the water activity is too low?



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How do they survive?

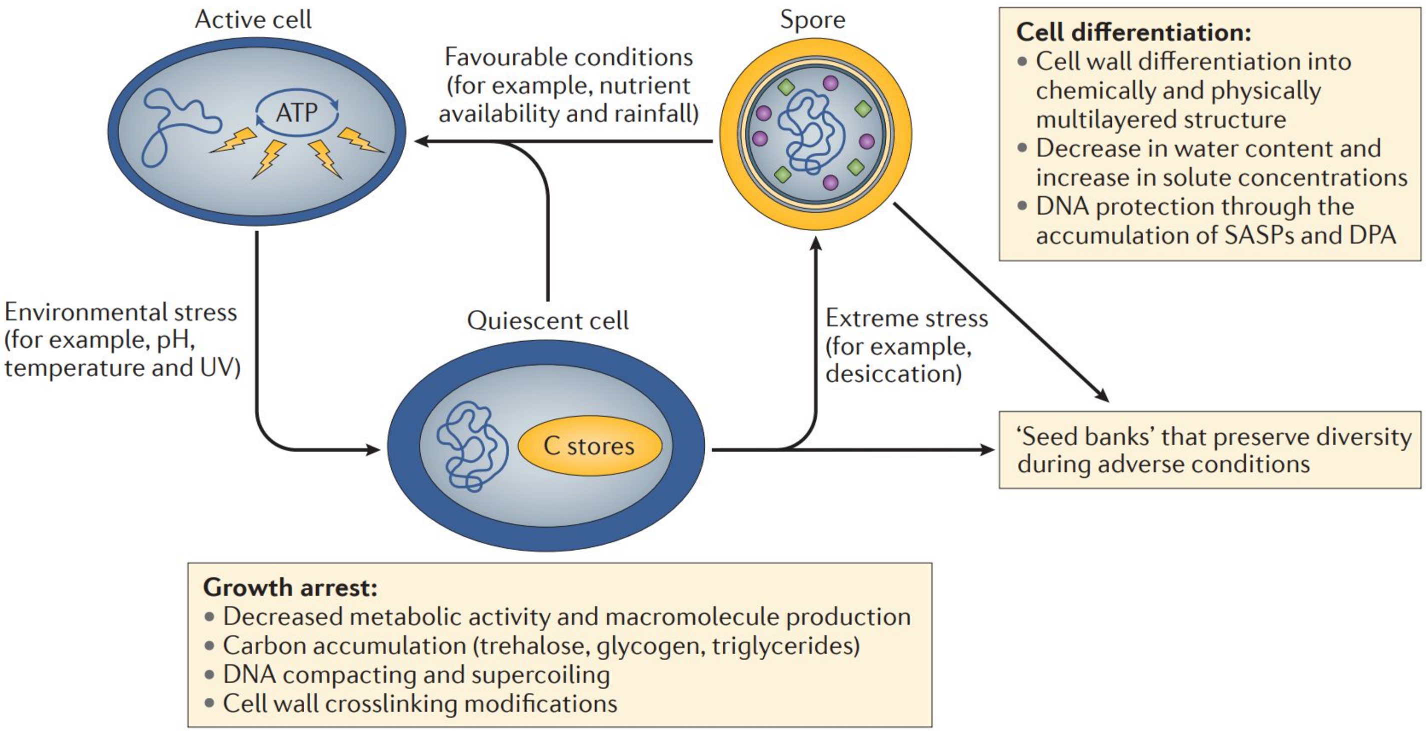
To counteract the negative morphological, physiological and biochemical consequences of desiccation, xerotolerant microorganisms have developed a broad range of adaptive strategies.

How do they survive?

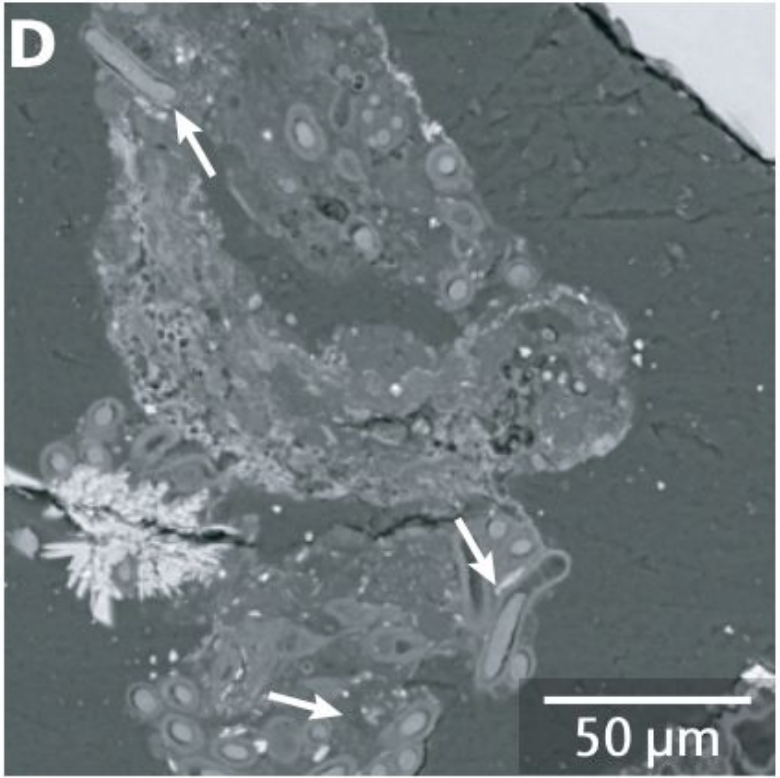
To counteract the negative morphological, physiological and biochemical consequences of desiccation, xerotolerant microorganisms have developed a broad range of adaptive strategies.

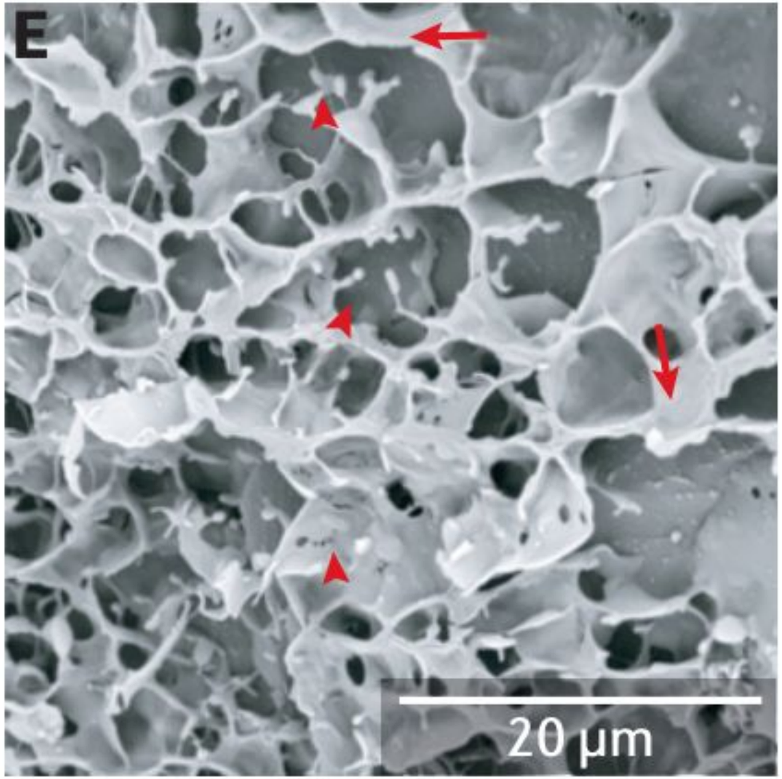
They have evolved a series of **behavioural, physiological and molecular** mechanisms

Behavioural adaptations: Dormancy and sporulation

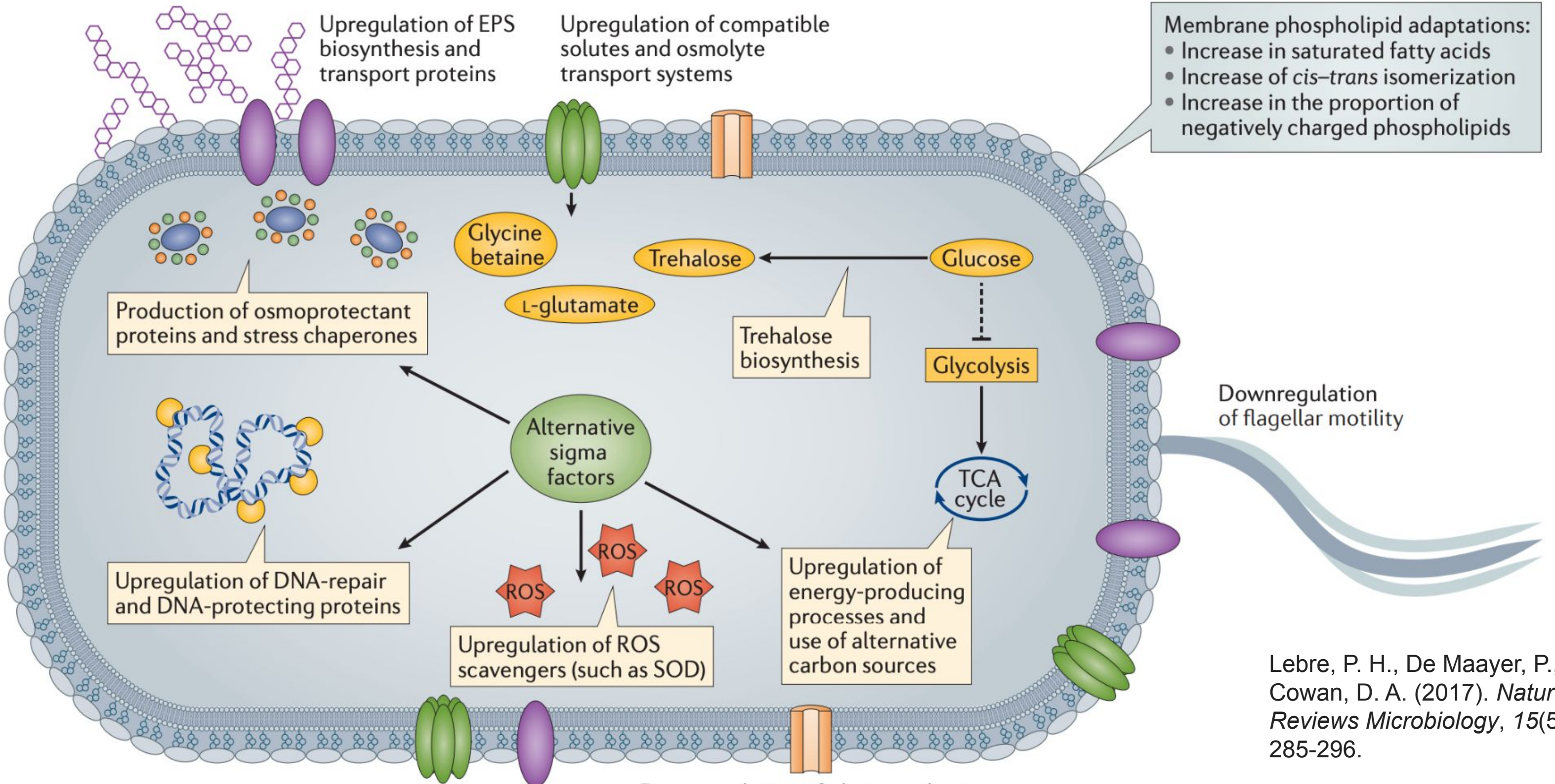


Behavioural adaptations: EPS and biofilm formation





Physiological adaptations: Cell membrane adaptations, accumulation and biosynthesis of compatible solutes



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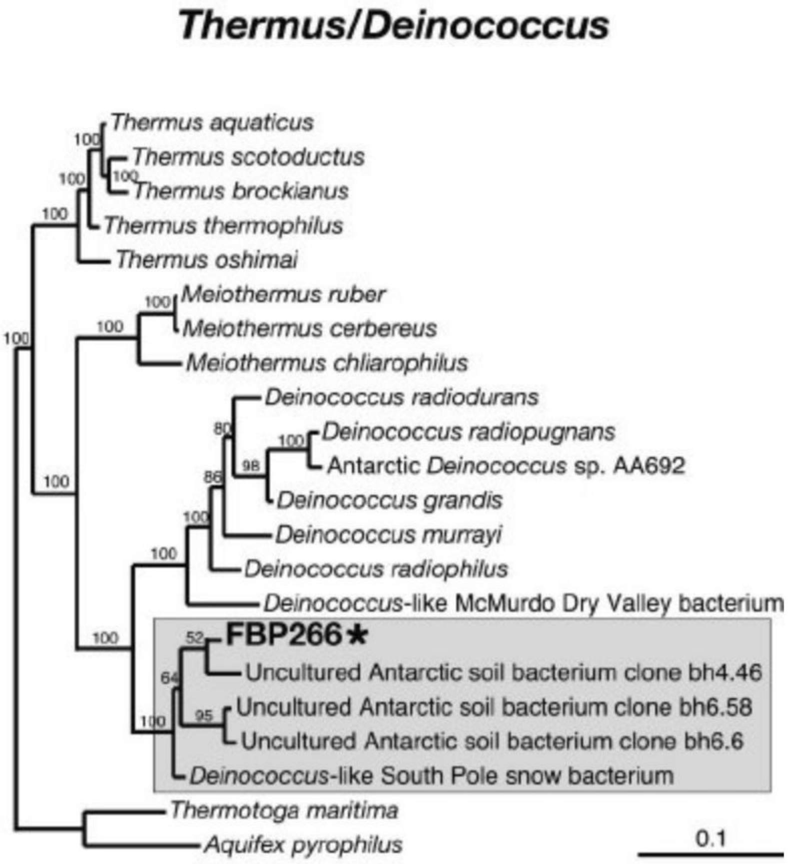
Metabolic and molecular adaptations

- They can differentially upregulate and downregulate various metabolic pathways, which can lead to a shift from anabolic to catabolic metabolism

- They can express a range of proteins to counteract the effects of low water activity es. Late embryogenesis abundant (LEA)

Thermus/Deinococcus phylum



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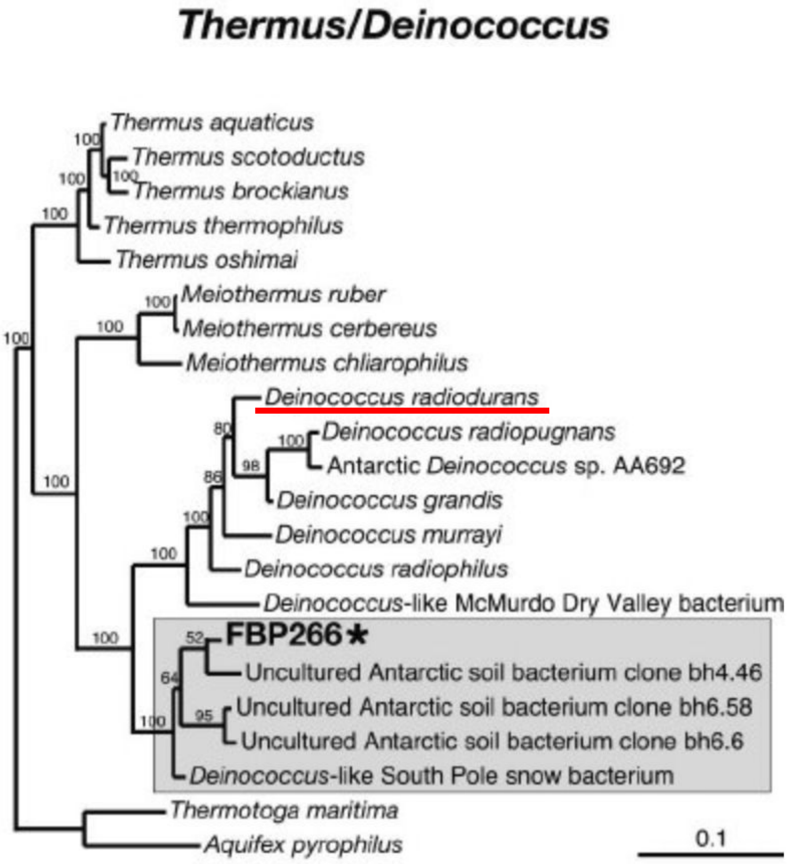

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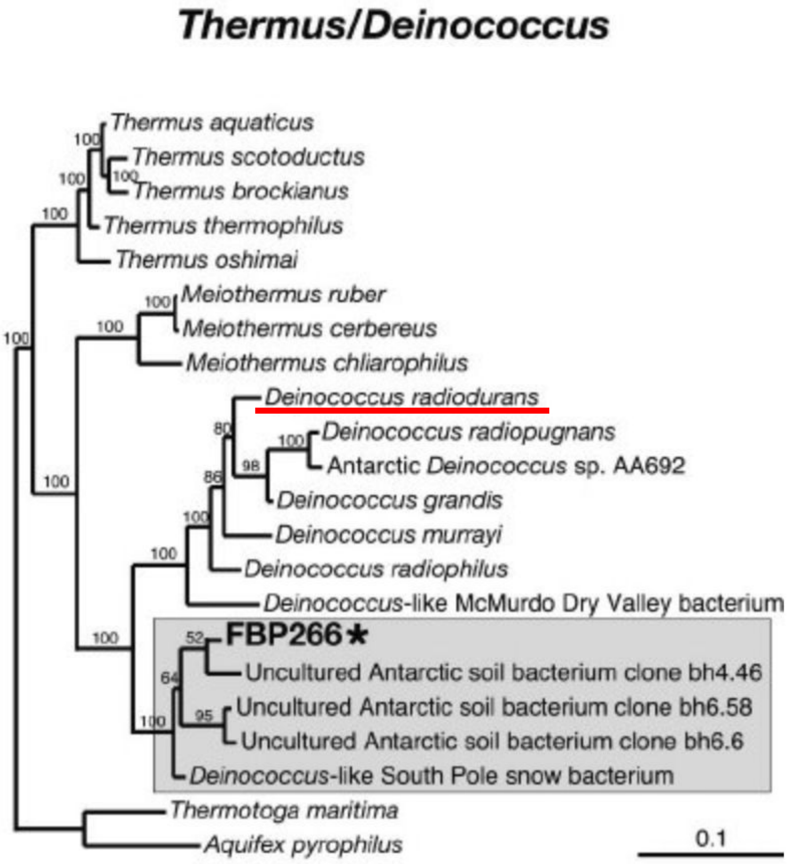
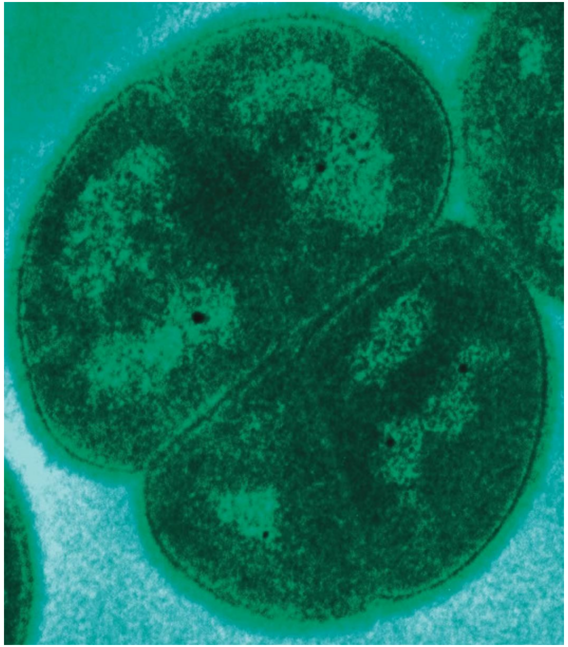


Deinococcus radiodurans



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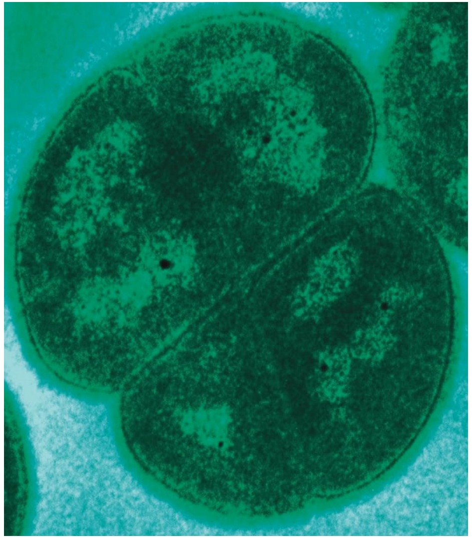
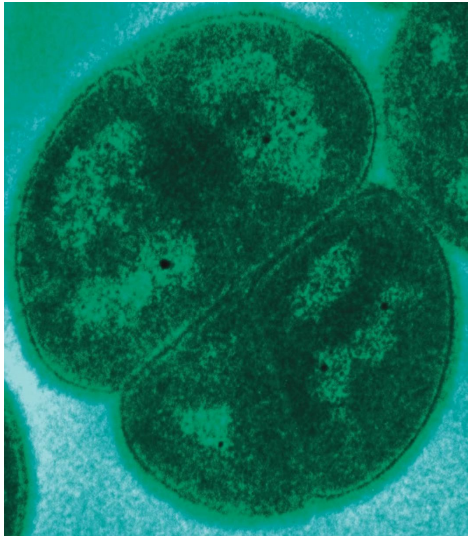


It is a **polyextremophile**:

- one of the most **radiation-resistant** organisms known
- It can survive cold, dehydration and vacuum

Deinococcus radiodurans



It is a **polyextremophile**:

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- It can survive cold, dehydration and vacuum

- Humans cannot survive a whole-body exposure of 10 Gy
- 200 Gy is lethal for most bacteria
- Deinococcus radiodurans can survive acute exposures of 15,000 Gy and chronic exposures of 60 Gy/h

Radiation-resistance

UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

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UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

Radiation-resistance limits

- *Thermococcus gammatolerans* EJ3 and *Deinococcus hohokamensis* have been shown to resist up to 30 kGy of γ-radiation

Radiation-resistance

UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

Radiation-resistance limits

- *Thermococcus gammatolerans* EJ3 and *Deinococcus hohokamensis* have been shown to resist up to 30 kGy of γ-radiation
- *Psychrobacter pacificensis* L0S3S-03b can resist up to 100–1000 J/m2 of UV254

Radiation-resistance evolution

There are no naturally occurring environments known that result in exposures exceeding 400 mGy per years

How it has evolved?

Radiations

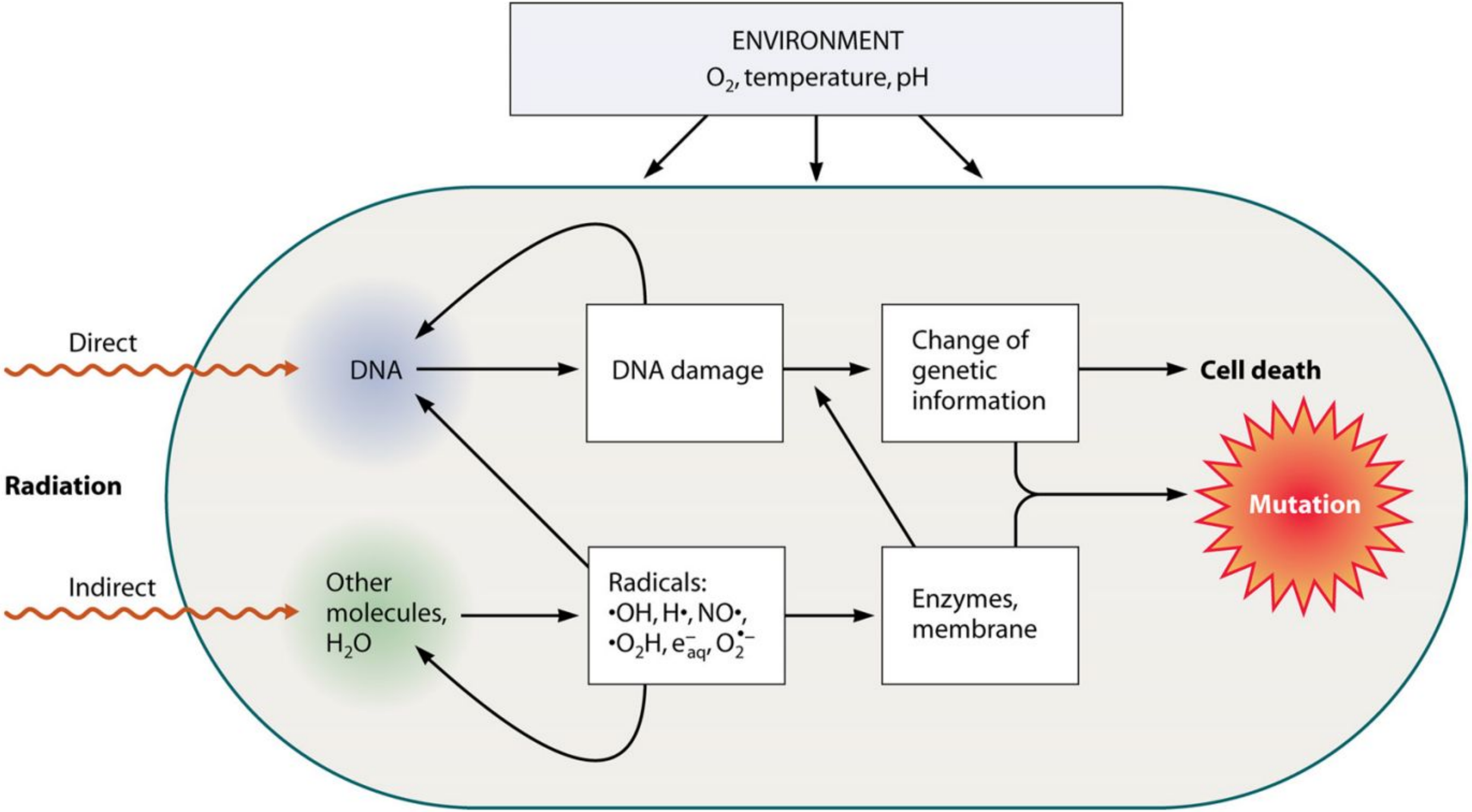
Other stresses like desiccation

They cause similar damages

The effects of ionizing radiations

The effects of ionizing radiation on living cells include:
the formation of large numbers of **ROS, DNA SSBs, DNA DSBs** and extensive **base modifications**; combinations of all of these induce the so-called **complex (clustered) DNA damage**

A dose of 6 kGy is expected to induce approximately **200 DSBs, over 3000 SSBs** and many more sites of DNA base lesions per D. radiodurans' genome. In addition, no significant differences have been found in genomic damage accumulation between radioresistant and radiosensitive bacteria.



Horneck, G., Klaus, D. M., & Mancinelli, R. L. (2010). *Microbiology and Molecular Biology Reviews*, *74*(1), 121-156.

Survival strategies

They need two main response systems:

Survival strategies

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- DNA and protein protection mechanisms

Survival strategies

They need two main response systems:

- DNA and protein protection mechanisms
- Efficient DNA repair mechanisms to restore genome functionality

Survival strategies

- **Small non-coding RNAs** (sRNAs) regulate DNA repair gene expression
- Genome redundancy
- Novel two-steps DNA repair processes

Published: 27 January 2009

A new perspective on radiation resistance based on *Deinococcus radiodurans*

Michael J. Daly

Nature Reviews Microbiology **7**, 237–245 (2009) | Cite this article

Abstract

In classical models of radiation toxicity, DNA is the molecule that is most affected by ionizing radiation (IR). However, recent data show that the amount of protein damage caused during irradiation of bacteria is better related to survival than to DNA damage. In this Opinion article, a new model is presented in which proteins are the most important target in the hierarchy of macromolecules affected by IR. A first line of defence against IR in extremely radiation-resistant bacteria might be the accumulation of manganese complexes, which can prevent the production of iron-dependent reactive oxygen species. This would allow an irradiated cell to protect sufficient enzymatic activity needed to repair DNA and survive.

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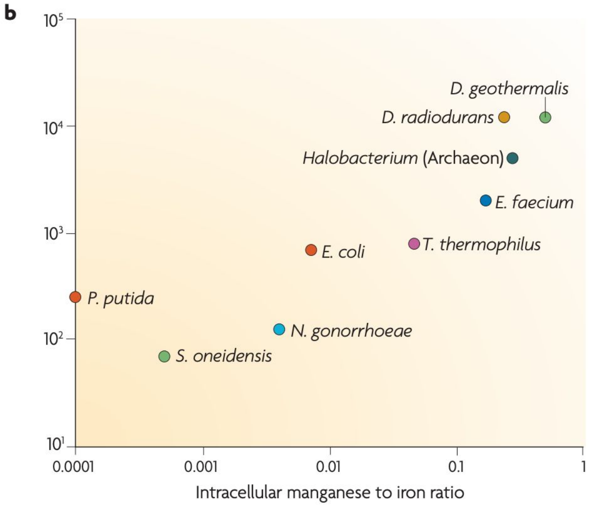

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A new perspective on radiation resistance based on *Deinococcus radiodurans*

Michael J. Daly

Nature Reviews Microbiology **7**, 237–245 (2009) | Cite this article

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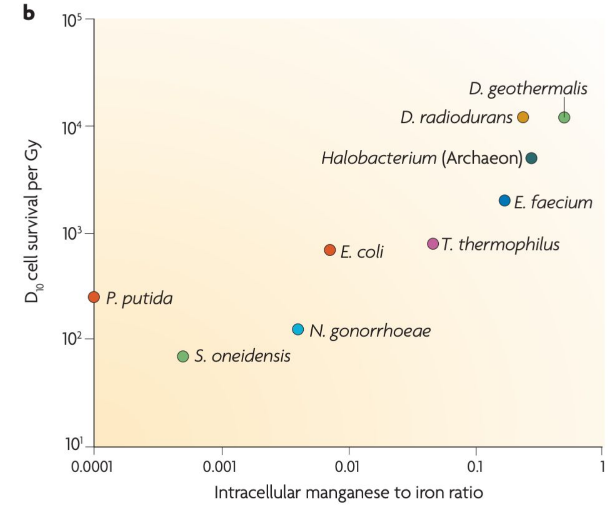

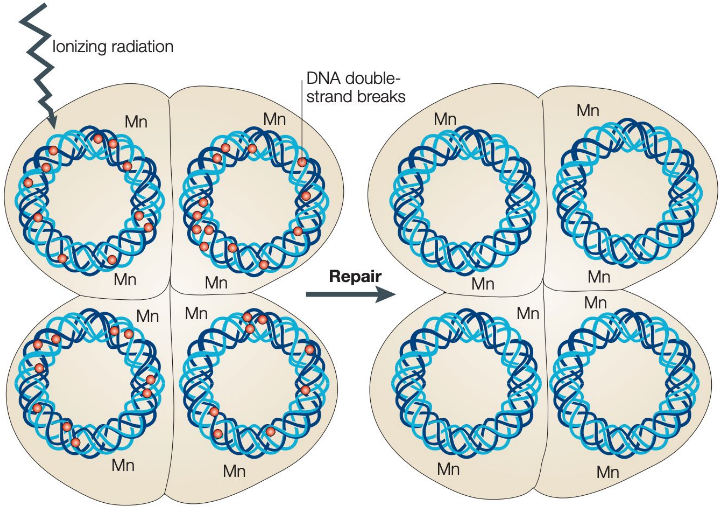

Where?

Table 1 Radiation-resistant extremophiles isolated from different types of environments

Organism	Environment	Radiation	Reference
Cellulosimicrobium cellulans UVP1; *Bacillus pumilus* UVP4	Elevated land	UVR-type C	Gabani et al. (2012a)
B. pumilus SAFR-032	International Space Station	UVR	Vaishampayan et al. (2012)
Bacillus subtilis	Earth's megnetosphere	UVR	Nicholson et al. (2011)
B. subtilis HA101	Simulated Martian environment	UVR	Kerney and Schuerger (2011)
Hymenobacter xinjiangensis	Desert	UVR and gamma	Zhang et al. (2007a)
Rubrobacter radiotolerance	Unknown	Gamma	Terato et al. (2011)
Sphingomonas sp. RB2256	Unknown	UVR-type B	Joux et al. (1999)
Chroococcidiopsis sp.	Desert and hypersaline	X-ray	Billi et al. (2000)
Halobacterium salinarum	Unknown	X-ray	Robinson et al. (2011)
*Deinococcus radiodurans*R1	Canned meat	X-ray, UVR, and gamma	Shukla et al. (2007)
Bacillus megaterium	Lake	UVR	Zenoff et al. (2006)
Staphylococcus saprophyticus	Lake	UVR	Zenoff et al. (2006)
Acinetobacter sp. Ver3, Ver5, and Ver7	Lake	UVR-type B	Di Capua et al. (2011)
Streptomyces radiopugnans	Radiation–pollution	Gamma	Mao et al. (2007)

Gabani, P., & Singh, O. V. (2013).. *Applied microbiology and biotechnology*, *97*(3), 993-1004.

Table 2 | **Species of ionizing-radiation-resistant bacteria**

Species	**Representative D_{10} value***	**Phylum**	**Refs**
Methylobacterium radiotolerans	1,000 Gray	α-Proteobacteria	80,81
Kocuria rosea	2,000 Gray	Actinobacteria	4,8
Acinetobacter radioresistens	2,000 Gray	γ-Proteobacteria	82
Kineococcus radiotolerans	2,000 Gray	Actinobacteria	83
Hymenobacter actinosclerus	3,500 Gray	Flexibacter–Cytophaga–Bacteroides	84
Chroococcidiopsis spp.	4,000 Gray	Cyanobacteria	11
Rubrobacter xylanophilus	5,500 Gray	Actinobacteria	85
Deinococcus radiodurans R1	10,000 Gray	Deinococcus–Thermus	86
Rubrobacter radiotolerans	11,000 Gray	Actinobacteria	85

Cox, M. M., & Battista, J. R. (2005). Deinococcus radiodurans—the consummate survivor. *Nature Reviews Microbiology*, *3*(11), 882-892.

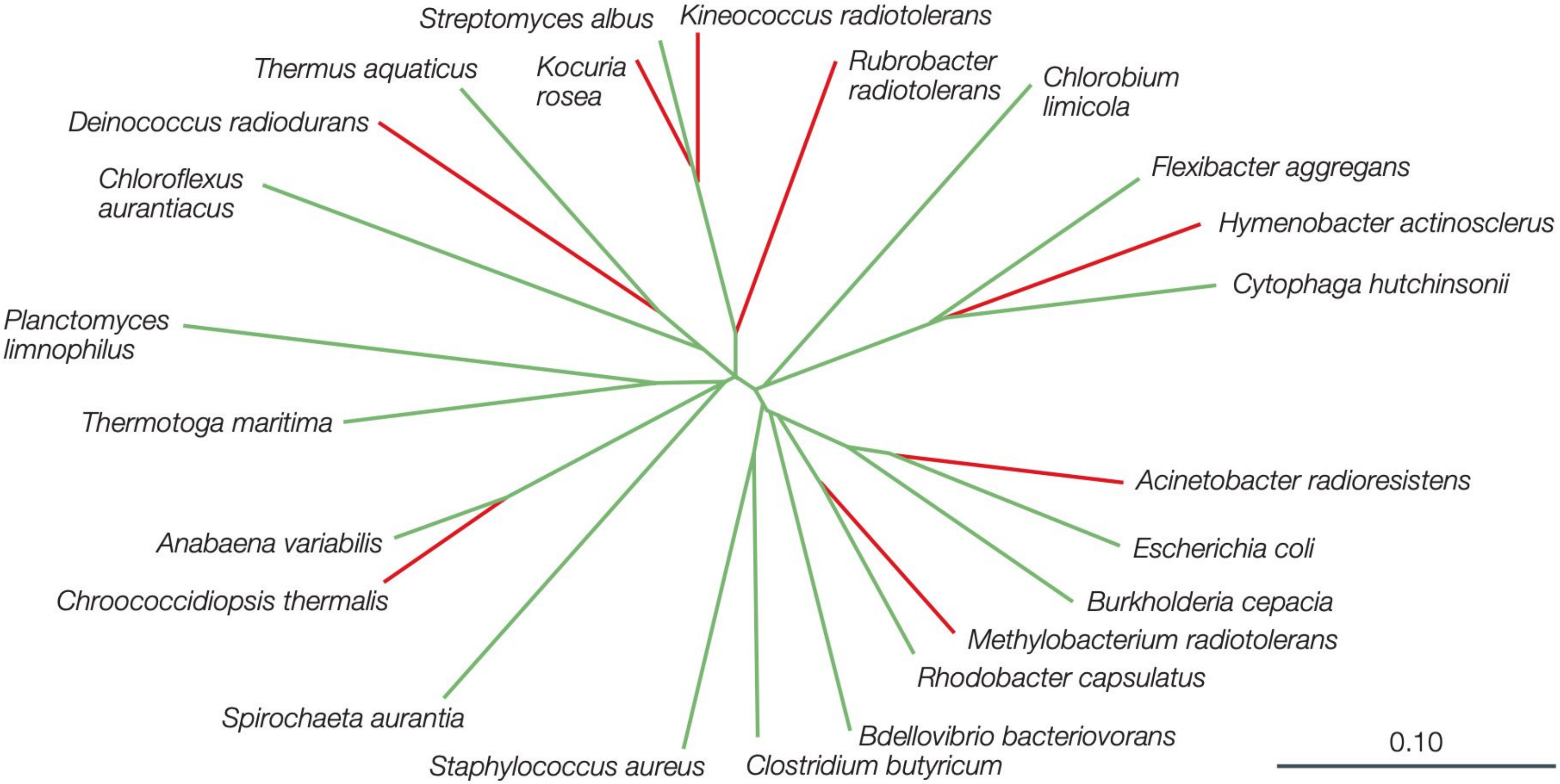

Figure 2 | **A 16S-rRNA-gene-sequence-based phylogeny of the main lineages of the domain Bacteria.** The branches in red are those in which ionizing-radiation-resistant taxa have been described. The scale bar represents 10 inferred nucleotide substitutions per 100 nucleotides.

Two hypotheses

Radioresistance is a vestige of DNA-repair mechanisms that were present in ancestral species retained in those organisms that continue to require this phenotype

This phenotype has arisen in unrelated species through horizontal gene transfer, or possibly convergent evolution

Applications

- Bioremediation of nuclear waste
- D. radiodurans has been proven effective in bioremediation of heavy metals from acidic and neutral water
- Several UVR-protective compounds have been isolated from UVR-resistant extremophiles

Reads

- Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, *15*(5), 285-296.
- Daly, M. J. (2009). A new perspective on radiation resistance based on Deinococcus radiodurans. *Nature Reviews Microbiology*, *7*(3), 237-245.
- Gabani, P., & Singh, O. V. (2013). Radiation-resistant extremophiles and their potential in biotechnology and therapeutics. *Applied microbiology and biotechnology*, *97*(3), 993-1004.
- Cox, M. M., & Battista, J. R. (2005). Deinococcus radiodurans—the consummate survivor. *Nature Reviews Microbiology*, *3*(11), 882-892.
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