MICROBIOLOGY OF EXTREME ENVIRONMENTS

Psychrophiles - the cold lovers

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GIOVANNELLI LAB

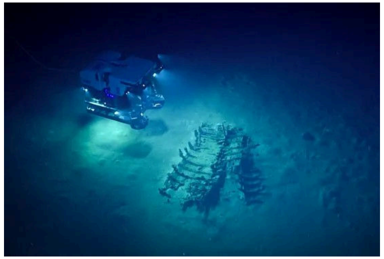
BIOLOGY OF EXTREME ENVIRONMENTS

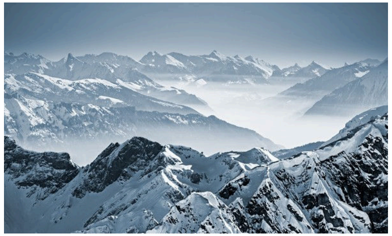
DIPARTIMENTO DI BIOLOGIA

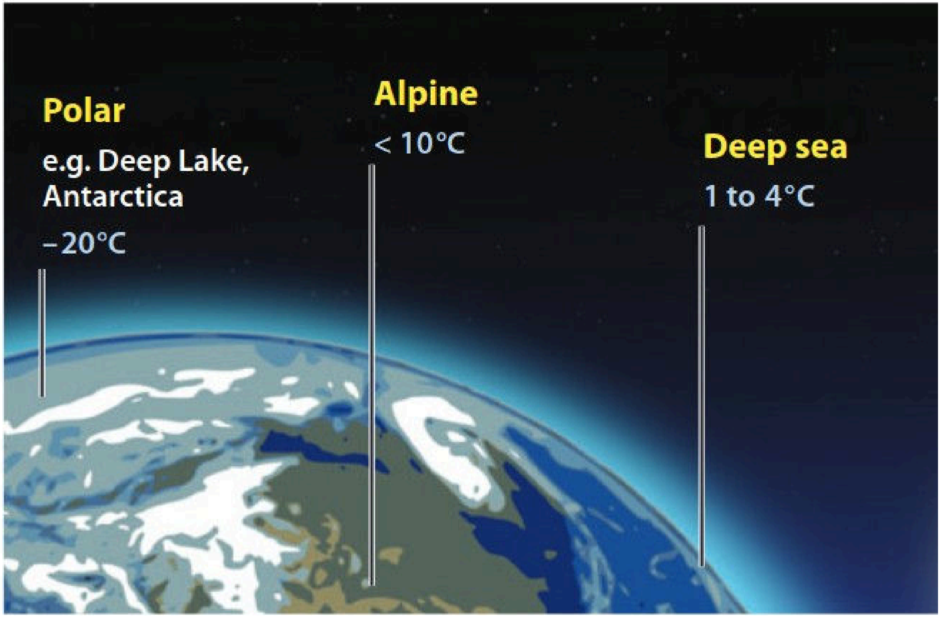
International Team sampling permafrost cores

The vast majority of our planet's surface is cold

70% of our planet's surface is **cold**

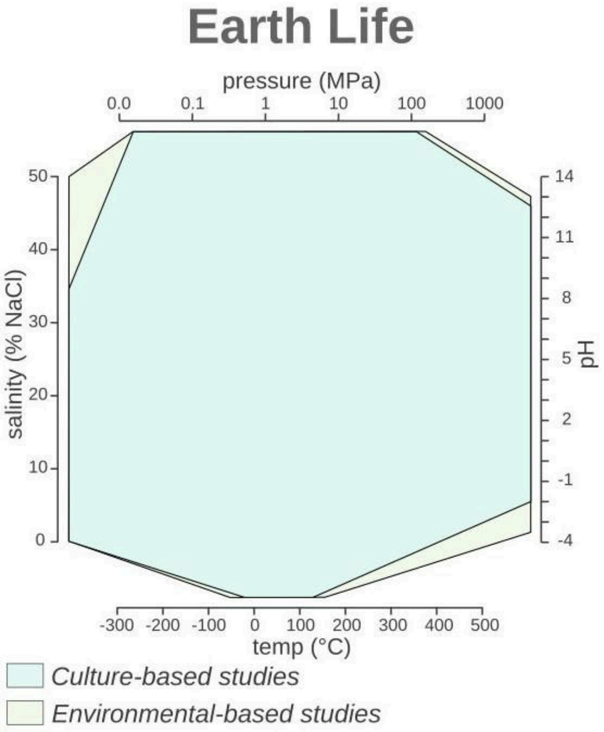






Most earth's ecosystems are exposed to **temperatures bellow 5°C**. The **cold biosphere** represents by far the **largest fraction** of the global biosphere

How cold, is too cold?
Life's limits on low temperatures

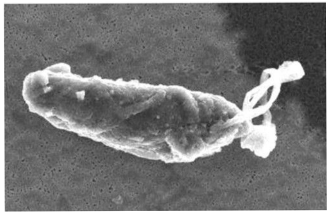


The **lowest temperature** ever recorded on our planet was **-98 °C**.

The **lowest temperature**, **microorganisms** have been observed to be **metabolic active** is **-28°C** (*Deinococcus geothermali*) in siberian permafrost

Cold environments
Who lives there?

There is a **big diversity** of organisms inhabiting and fully adapted to **cold** temperatures. Some examples:



Bacteria/Archaea

Fungi

Algae

Wim hoff

A **variety of physiotypes** have evolved to **successfully colonize cold** environments

Cold environments
Who lives there?

Psychrophilic vs Psychrotolerant

Psychrophilic

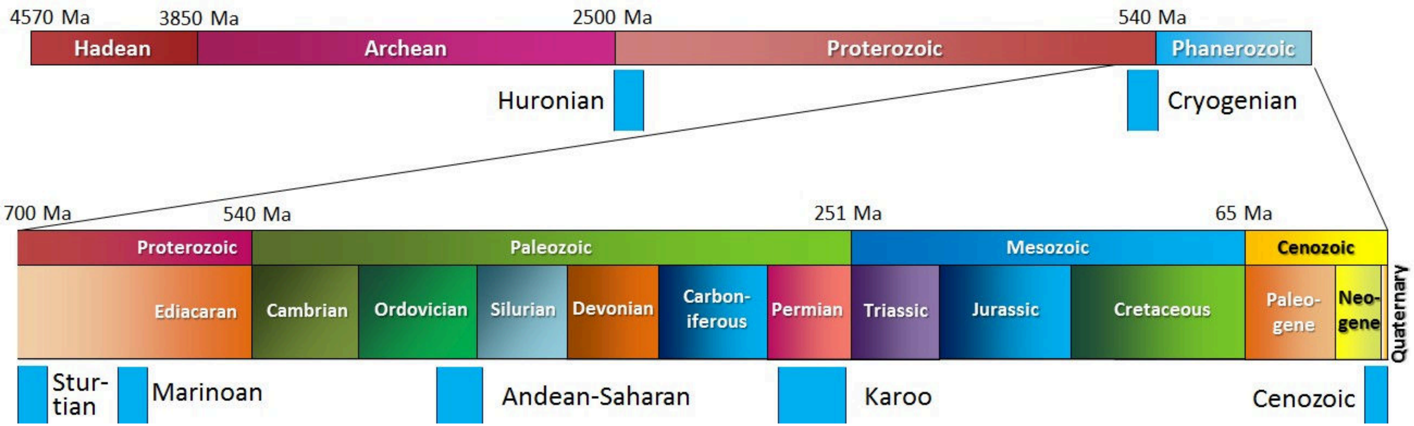
Fastest growth occurs at **<15°C** and it is not possible >20°C

Psychrotolerant

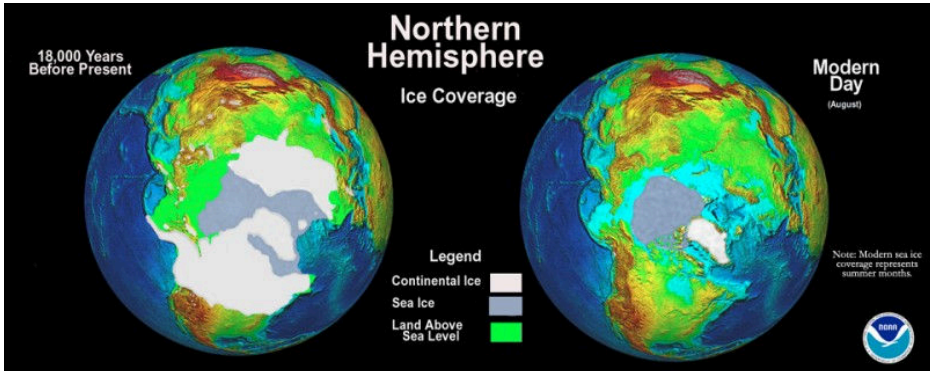
Capable of growing close to the freezing point of water, but fastest growth occurs at **>20°**

They have important roles in **nutrient** (carbon and nitrogen) **cycling** and **recycling**, and are the **beginning of polar food webs**,affecting all trophic levels

The evolution of our planet and the perseverance of cold environments



We can find **cold-adapted organisms** through different branches of the tree of life, suggesting that the **ability to adapt to cold evolved independently multiple times**



Because just cold is not enough
The poly-extremophilic psychrophiles

Psychrophiles are adapted **not only** to **low temperatures**, but also further environmental constraints.

Cold+pressure (baro-psychrophiles): Deep ocean and sediments.

Cold+salt (halo-psychrophiles): Sea ice, exposed to salt concentrations of several molar in brine veins.

Cold+radiation (Radio-psychrophiles): On the snow surface of glaciers and polar caps, exposed to strong ultra-violet radiation

Cold + oligothophy (troglo-psychrophiles): Rocks of antarctic deserts and alpine caves and cracks

Challenges for life in cold environments

Low-**temperatures**

Low-liquid **water availability**

Lowered enzyme **reaction rates**

Decreased membrane **fluidity**

Altered transport of nutrients and waste products

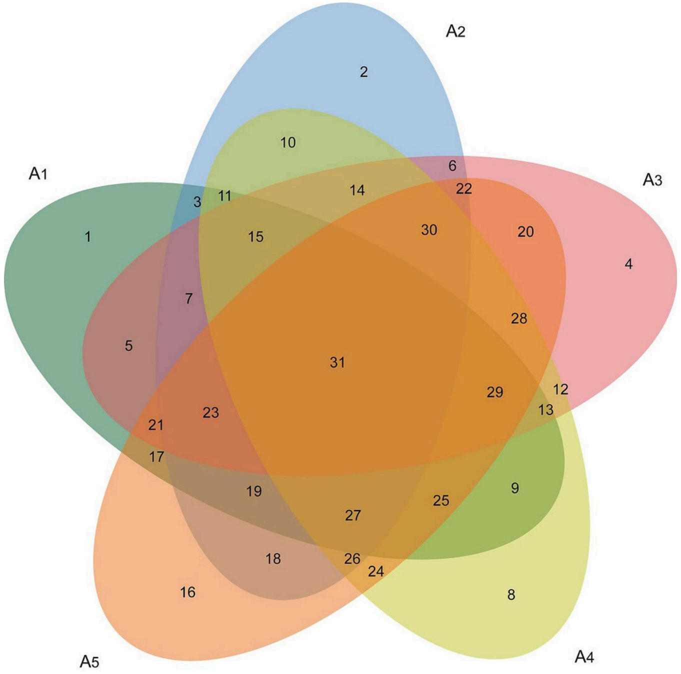
Intracellular crystalline **ice formations**

Inappropriate protein **folding**

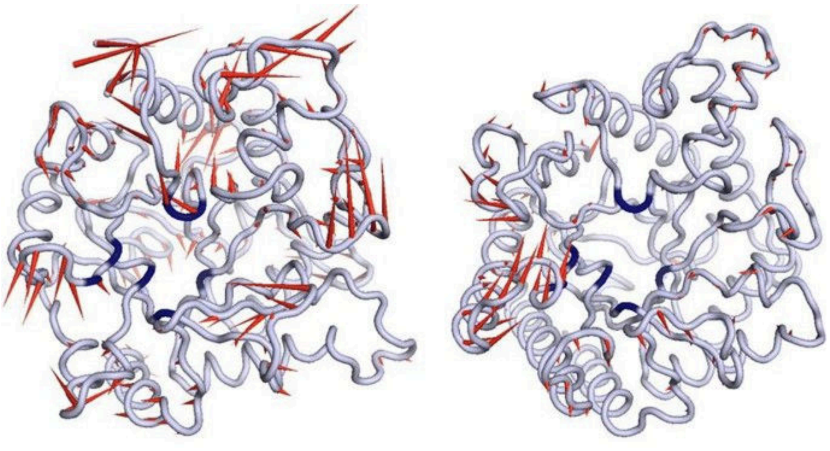
All of life's adaptations to cold environments need to address this challenges

*Cold-adapted organisms have **successfully evolved features**, genotypic and/or phenotypic, to **surmount the negative effects** of low-temperatures and to **enable growth** in these extreme environments*

On the search of adaptations: The power of comparison



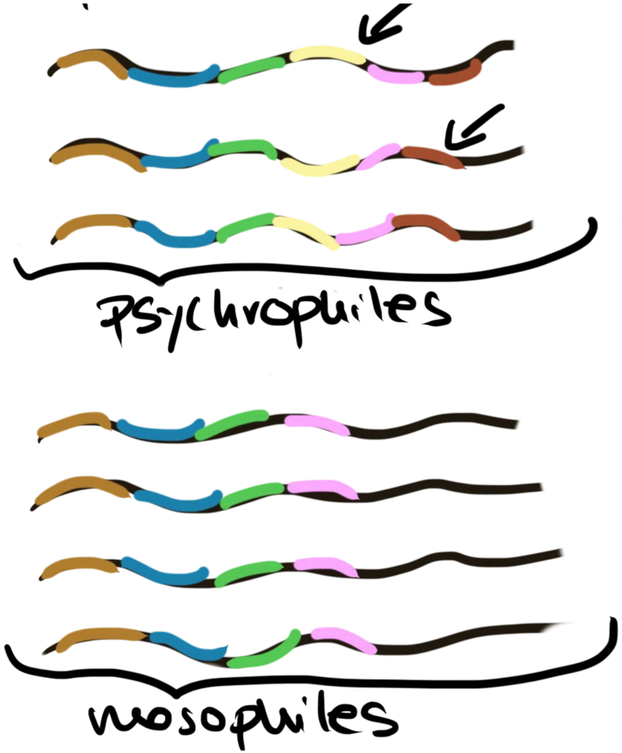
Physiological adaptations to growth temperatures can be identified by **comparing the properties** of microorganisms that grow naturally at different temperatures



Psychrophilic mannanase   Mesophilic mannanase

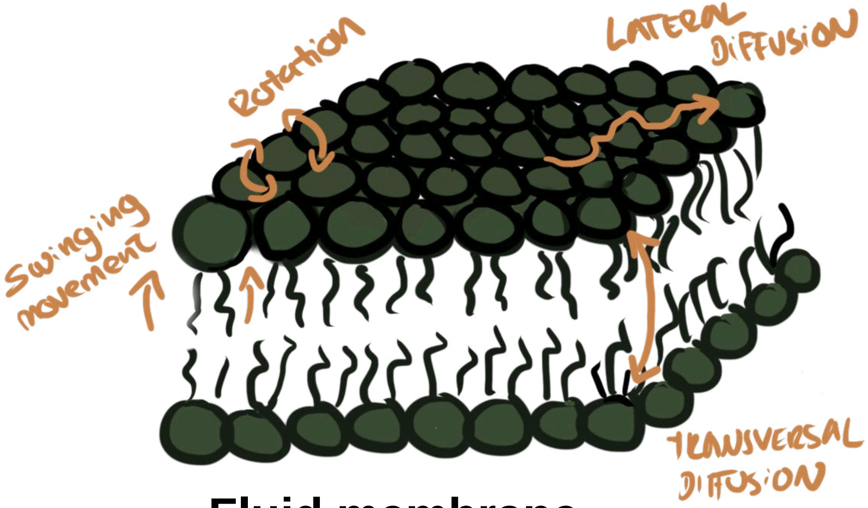
On the search of adaptations: The power of comparison

Comparative 'omics



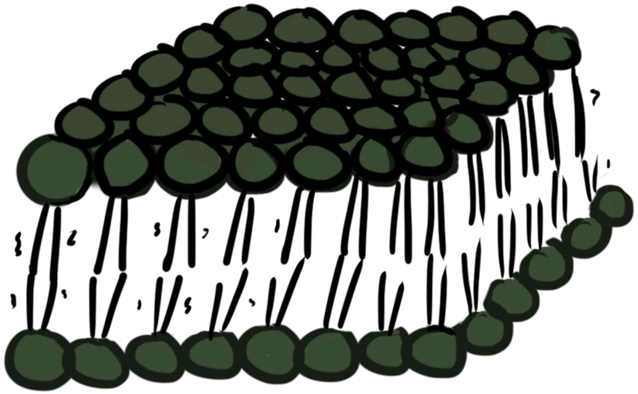
Many advances in understanding adaptive mechanisms **comes from studying genome sequences**. In addition to providing genome blue prints, genomes provide the basis of targeted and global functional studies (**proteomics and transcriptomics**)

On the adaptations to the cold: Maintaining membrane fluidity



Fluid membrane

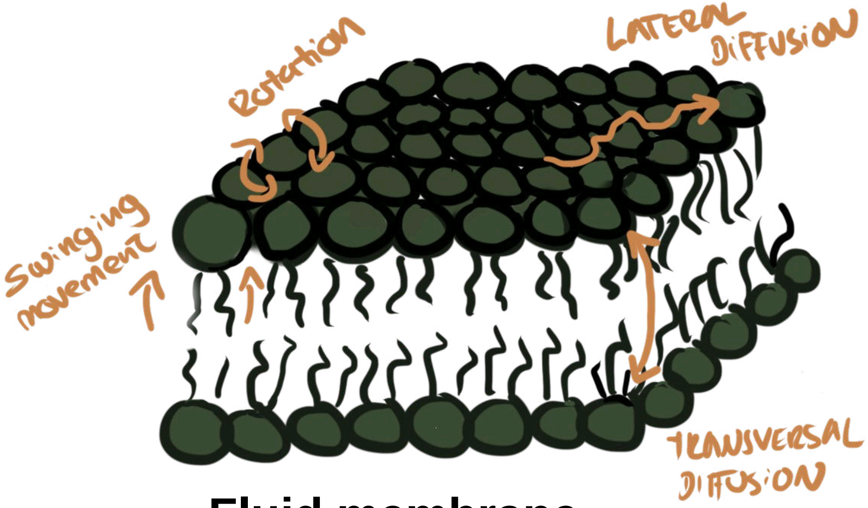
VS



Rigid membrane

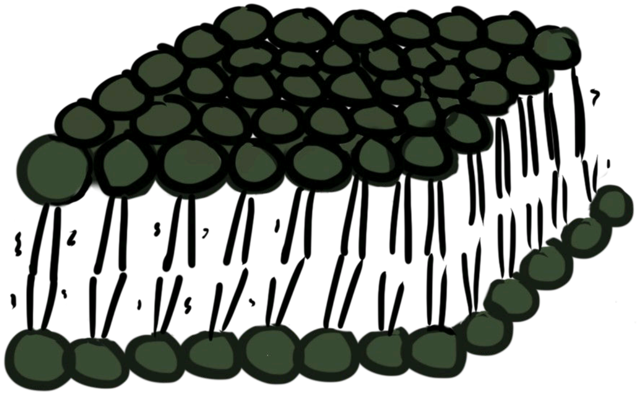
The problem: at freezing temperatures the membranes become **rigid**.

On the adaptations to the cold
Maintaining membrane fluidity



Fluid membrane

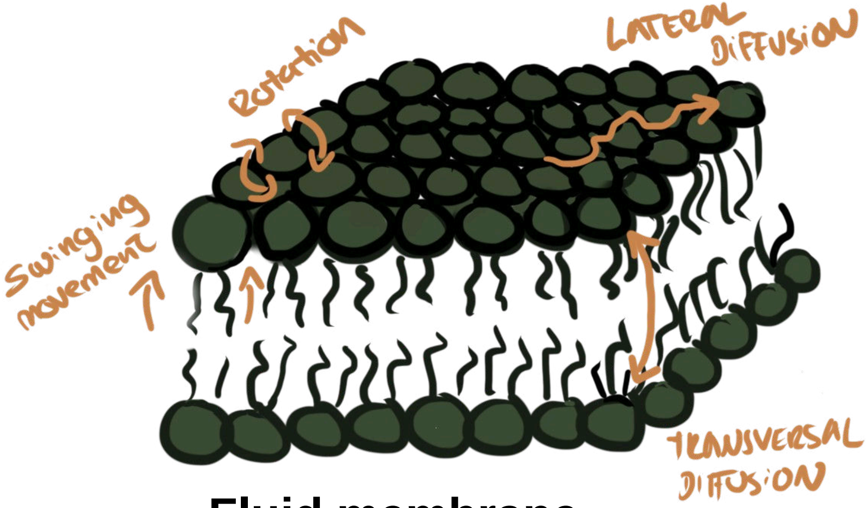
VS



Rigid membrane

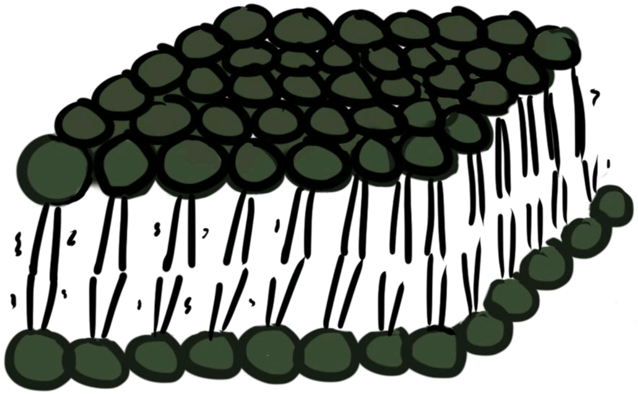
Why is it a problem: The lipid bi-layer must have the proper fluidity to **allow the permeability** properties and motions of essential membrane proteins.

On the adaptations to the cold
Maintaining membrane fluidity



Fluid membrane

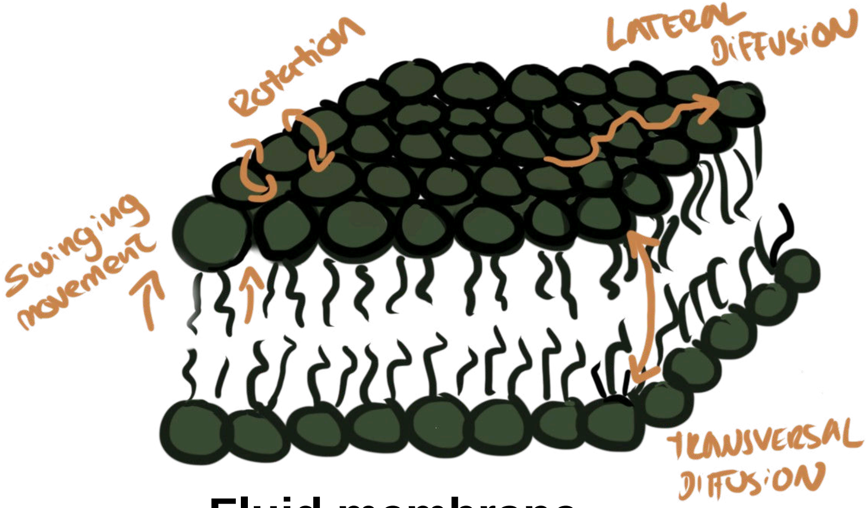
vs



Rigid membrane

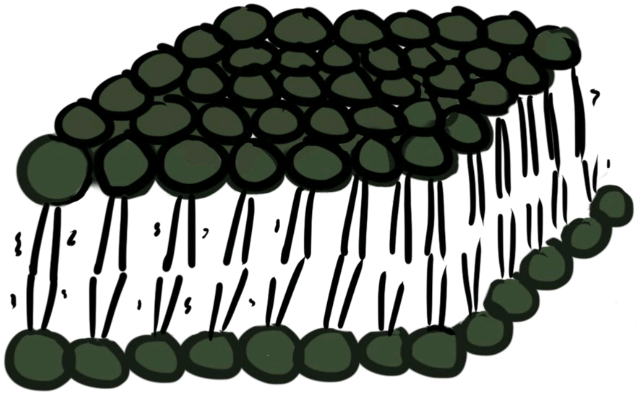
The consequence: Inactivates the function of certain trans-membrane proteins including carriers and transporters.

On the adaptations to the cold
Maintaining membrane fluidity



Fluid membrane

VS

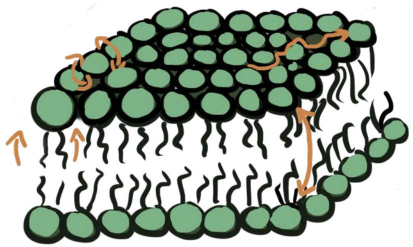


Rigid membrane

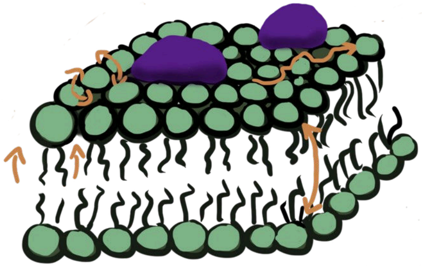
One of the most frequent adaptive strategies relates to the ability of the cell to regulate or modulate the fluidity of the membrane.

On the adaptations to the cold
Maintaining membrane fluidity

The solutions:



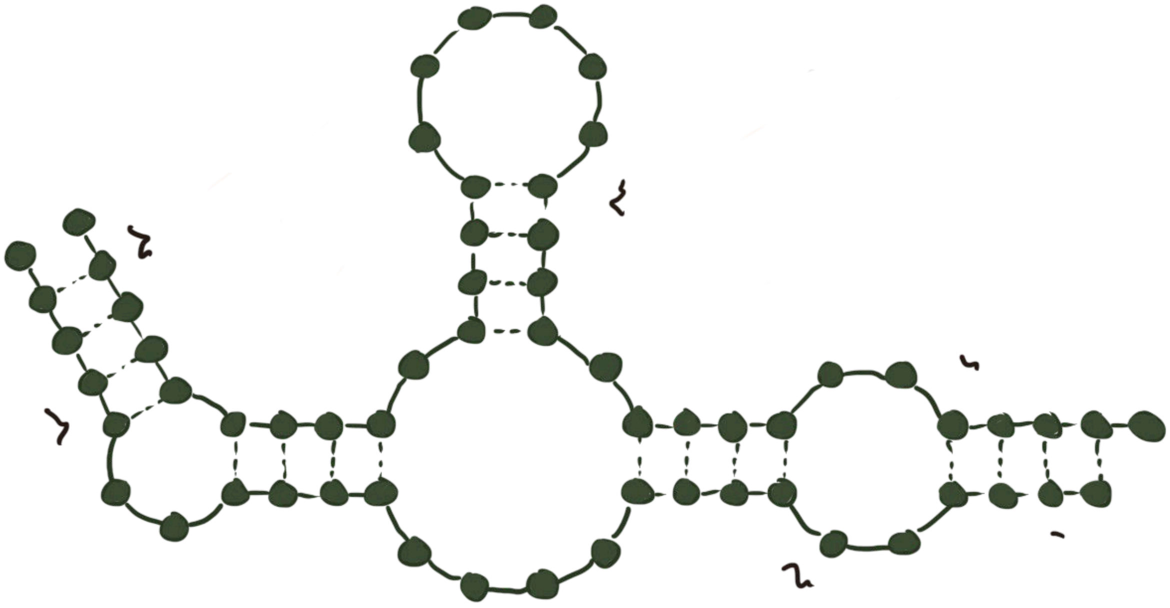
In order to **maintain membrane fluidity**, organisms utilize a combination of **changes in fatty acid composition,** including poly-unsaturated short-chain fatty acids.



Membrane bound caratenoids also modulate membrane fluidity. Polar pigments seem to be produced in larger amounts at low temperatures.

Metagenomic analysis found that **glacier ice is enriched for genes** involved in the **maintenance of membrane fluidity**. Membrane lipid changes appear to be a **generally conserved feature** of cellular adaptations to the cold

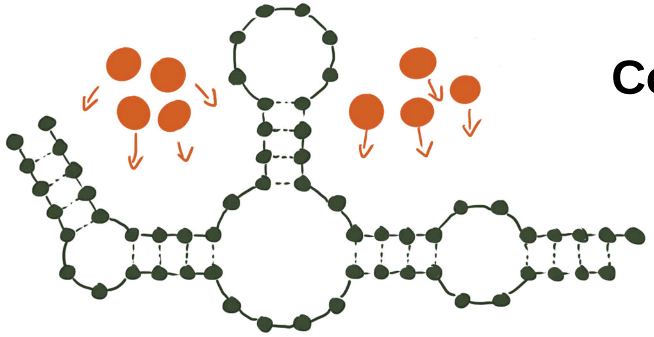
On the adaptations to the cold
Transcription and translation



The problem: Low temperatures can impede transcription and translation owing to the **increasing stability** of secondary structures of transcripts

On the adaptations to the cold
Transcription and translation

The solutions: Preventing or resolving inhibitory secondary structures of RNA



Cold shock response

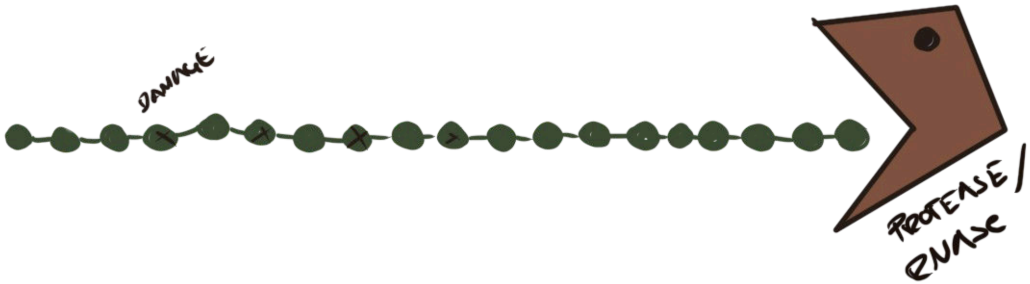
This task can be achieved with **Cold-shock proteins**, **DEAD-box helicases** and **Cold response TRAM domains**

The **most prominent response** of cells to cold shock

On the adaptations to the cold
Transcription and translation

The solutions Preventing or resolving inhibitory secondary structures of RNA

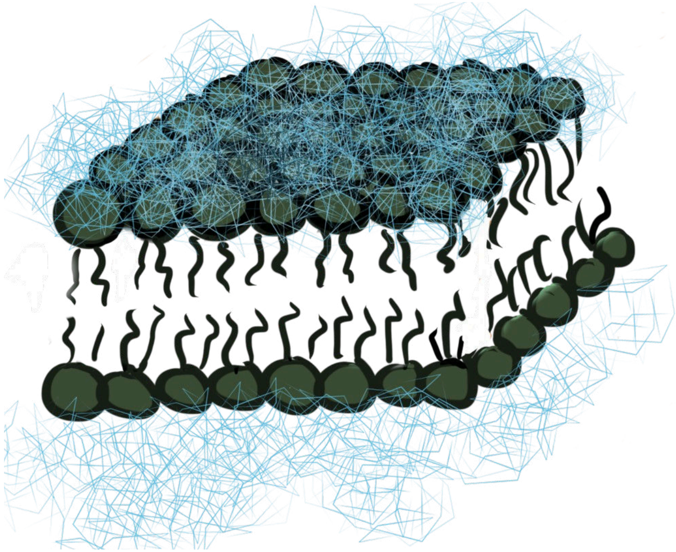
Up-regulation of enzymes involved in the **degradation of RNA**, including **RNAses** and **proteases**.



Enhanced quality control of irreparable damage RNA and proteins

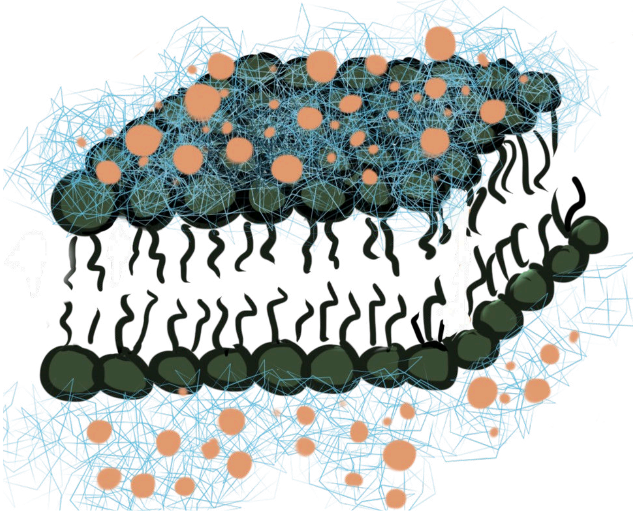
On the adaptations to the cold
Anti-freeze proteins

Problem: At low temperatures ice crystals began to form



On the adaptations to the cold
Anti-freeze proteins

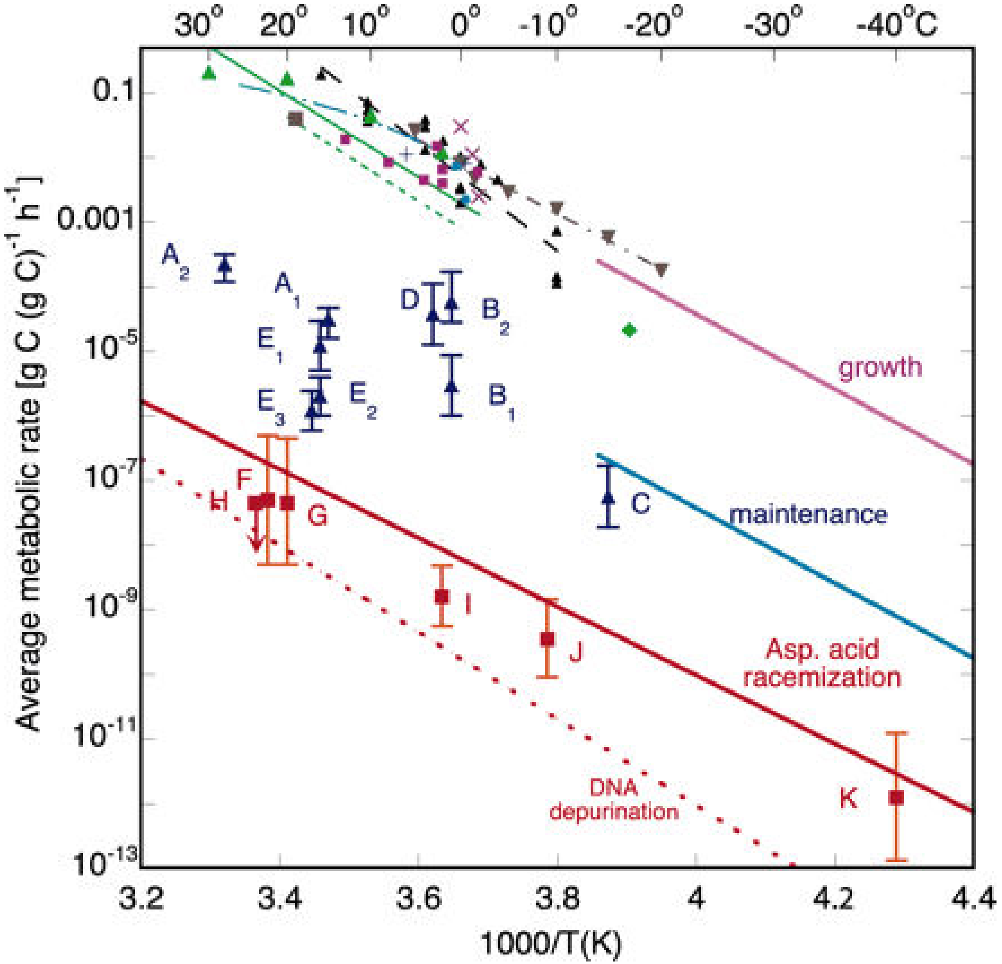
The solution: Production of **Anti-freezing proteins** and EPS aggregates



These enzymes have **affinity towards ice.** They can be classified based on weather they **promote avoidance to freezing**, or **promote tolerance to freeing**

Cryoprotectants are exopolymeric substances produce by high amounts to **prevent cold-induced aggregation of proteins**.

The major challenge of life at cold environments

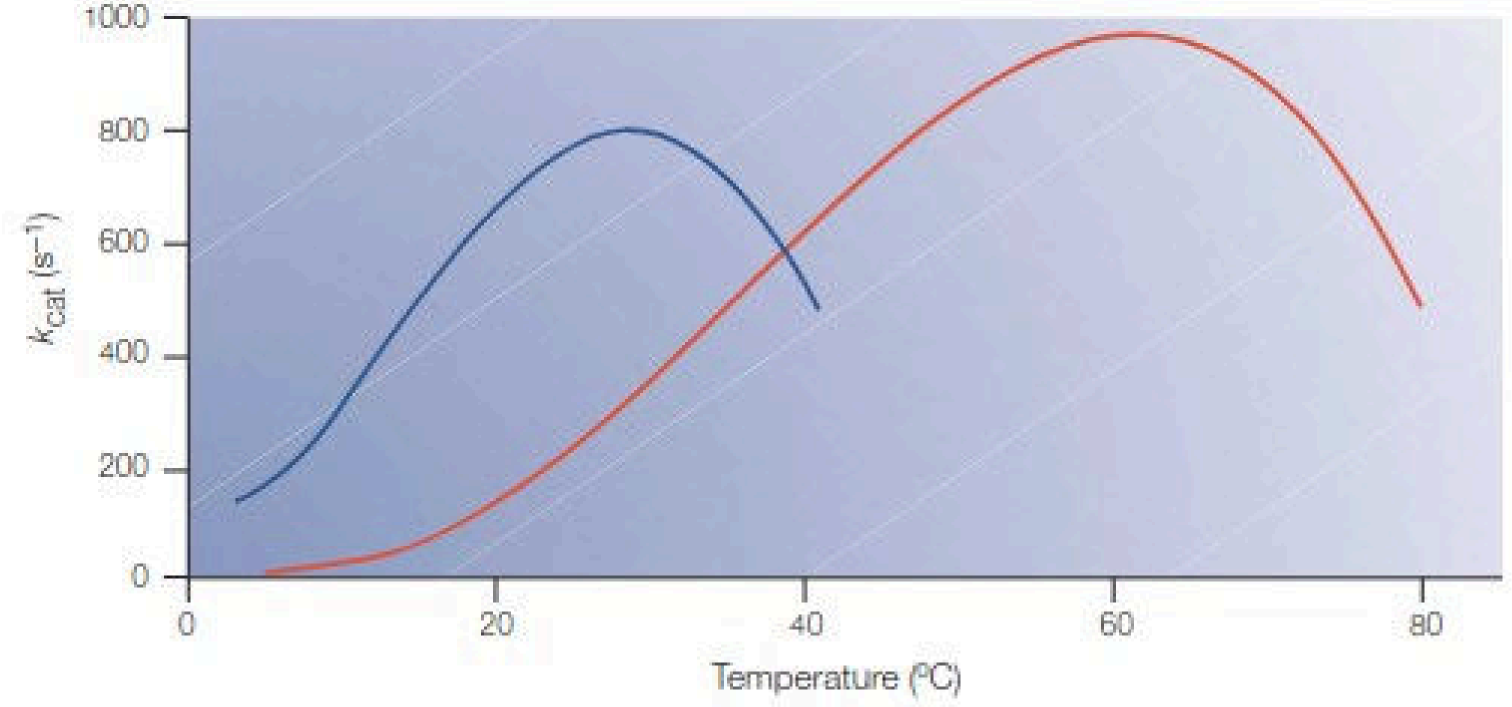


The problem: cold temperatures **lower chemical reaction rates (kinetics)**, which exponentially drop with decreasing temperature

The molecular adaptation of enzymes to compensate for reduced reaction rates considered a critical feature of cold-adapted microorganisms

The major challenge of life at cold environments

The solution: Cold-adapted proteins



The major challenge of life at cold environments

The solution: Cold-adapted proteins

The activity-stability-flexibility trade-off

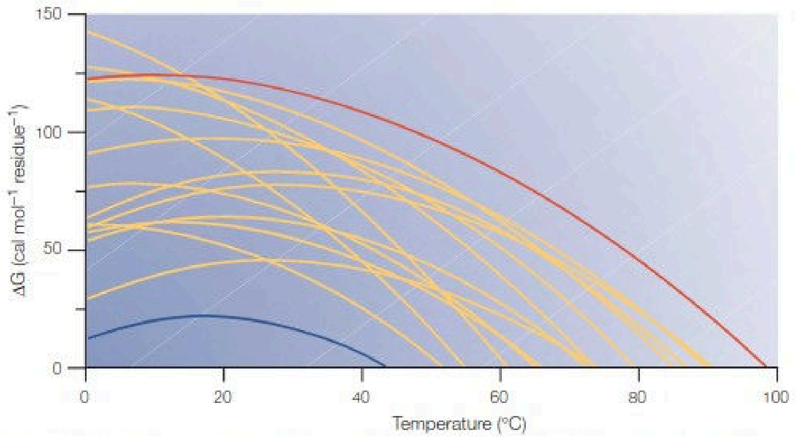
Psychrophilic proteins increase the **flexibility** of their **structure** to compensate the freezing effect of cold habitats. The **increased flexibility** can concern the **entire protein** or to be restricted to the regions responsible for **catalysis**

The **increased flexibility** is most due to **changes in amino-acid composition**, such as a **reduced** number of **proline** and **arginine** residues, and a **higher** number of **glycine** residues. Compared to mesophiles and thermophiles

The major challenge of life at cold environments

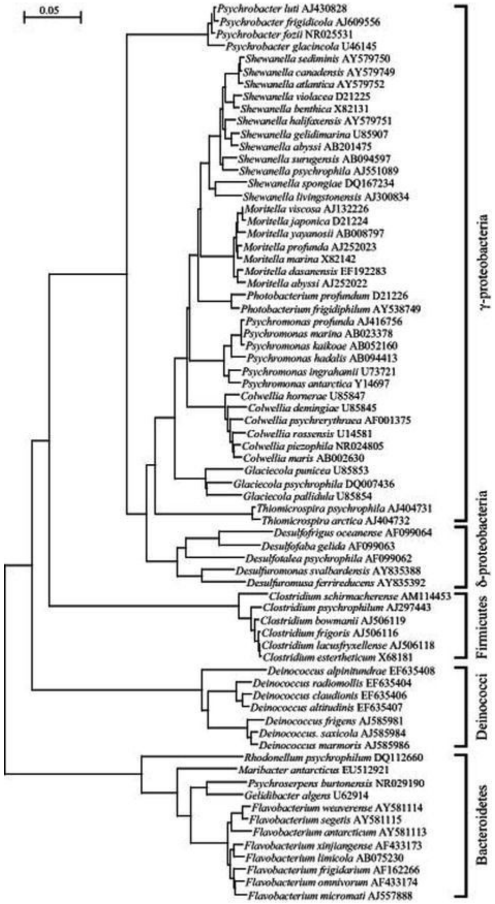
The solution: Cold-adapted proteins

The activity-stability-flexibility trade-off



The increase in **flexibility** is to **compensate the lower thermal energy** provided low temperature habitat, since it allows for a **good complementary with the substrate** at low energy cost. However, it comes with a price, which is the very **weak thermal activity** of psychrophilic proteins.

On the taxonomy of psychrophiles



Gammaproteobacteria

Genus:
Colwellia
Glaciecola
Moritella
Psychrobacter

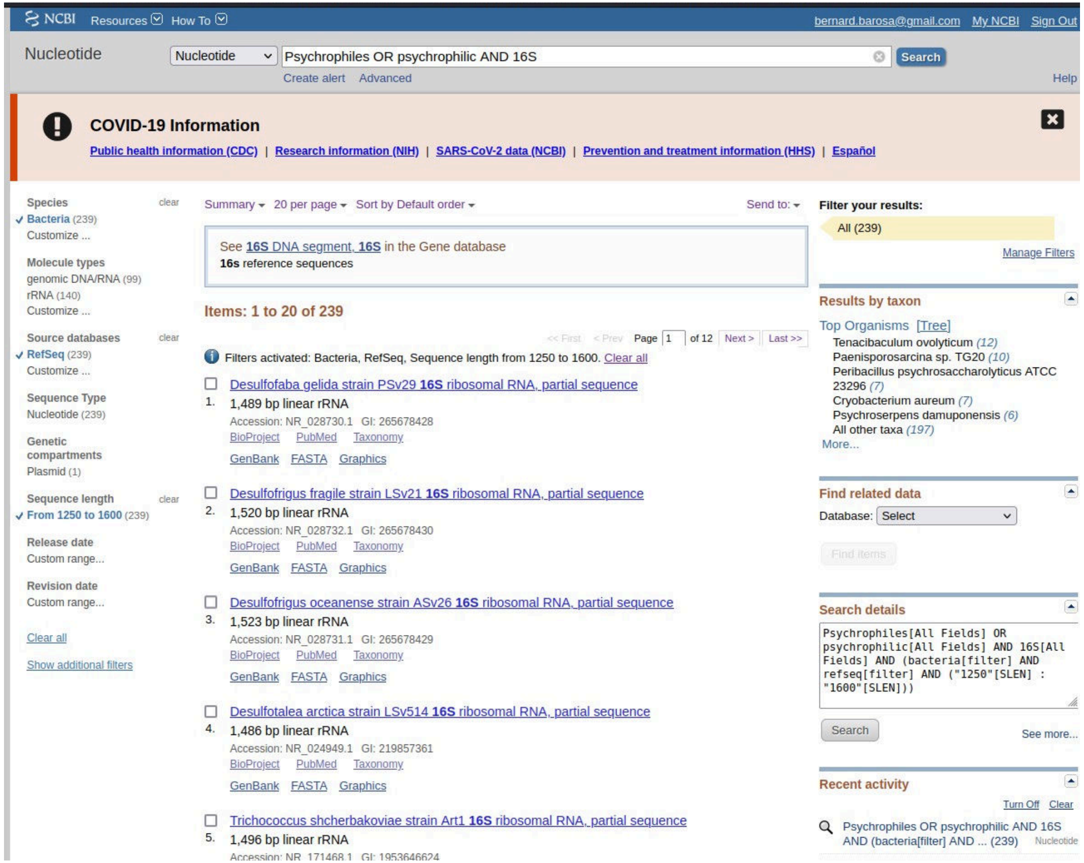
Deltaproteobacteria

Genus:
Desulfuromonas
Desulfuromusa
Desulfofrigus

Deinococci

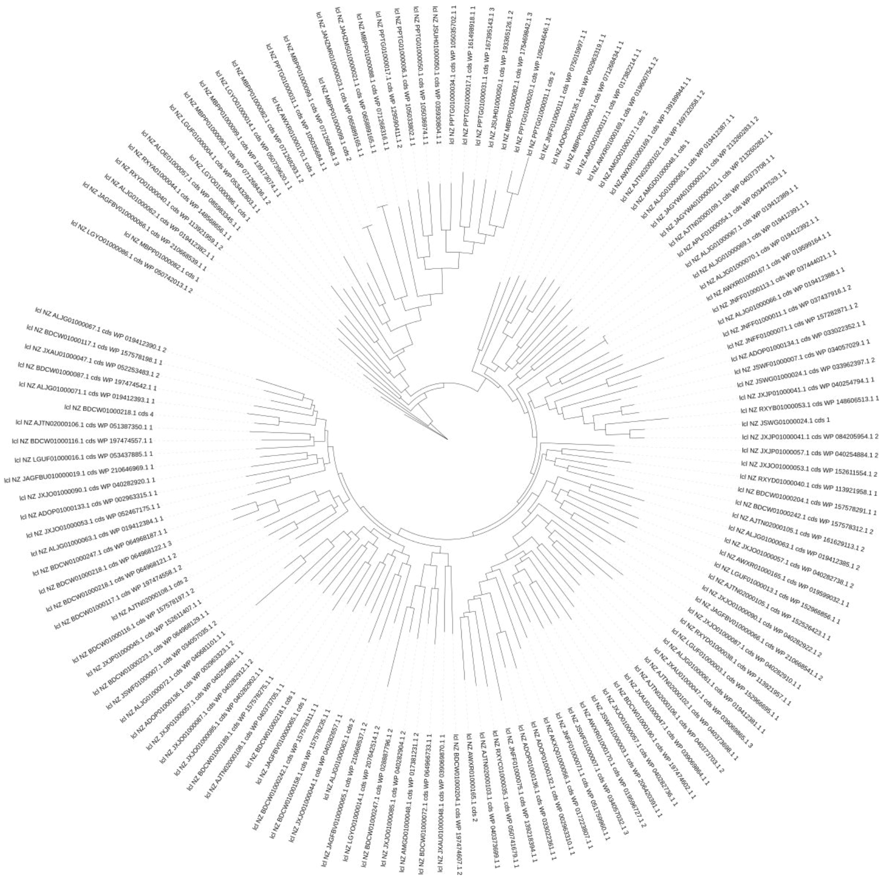
Genus:
Deinoccocus

On the taxonomy of psychrophiles



A quick 16S rRNA analysis

On the taxonomy of psychrophiles



A quick 16S rRNA analysis

Some Applications of Psychrophiles

1) **Bio-remediation** of polluted soils and waste waters in cold regions/ during winter

2) **Cold active pectinases** can help to **reduce viscosity and clarify** fruit juices at **low temperatures**

3) **Antifreeze proteins** can serve as **cryoprotectant** compared to the conventional process which uses liquid nitrogen

4) **Substantial energy savings** in large-scale processes that would not require the expensive heating of reactors

5) **Cold active hydrolases** can be used in detergents, to allow cold-washing

Psychrophiles and climate change: The deep purple project

The Darkening of the Greenland Ice Sheet

DEEP PURPLE

The deep purple project aims to understand why the Greenland Ice Sheet darkens during melt season due to pigmented ice algae blooms in the ice surface.

https://www.deeppurple-ercsyg.eu/

Psychrophiles and the the adaptations to permafrost

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Eight Metagenome-Assembled Genomes Provide Evidence for Microbial Adaptation in 20,000- to 1,000,000-Year-Old Siberian Permafrost

Katie Sipes,[a] Abraham Almatari,[a] Alexander Eddie,[a] Daniel Williams,[a] Elena Spirina,[b] Elizaveta Rivkina,[b] Renxing Liang,[c] Tullis C. Onstott,[c] Tatiana A. Vishnivetskaya,[a,b] Karen G. Lloyd[a]

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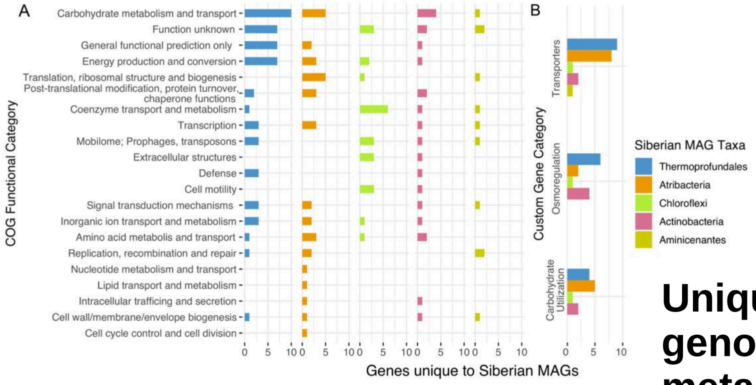
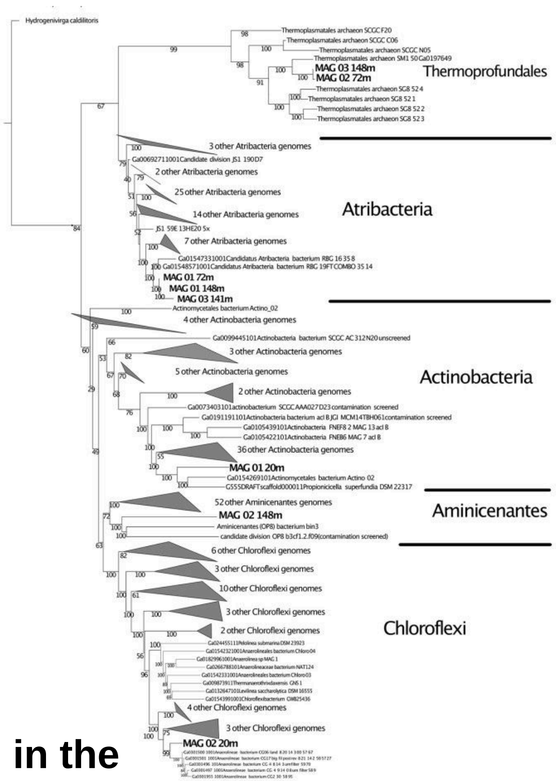
[b]Institute of Physicochemical and Biological Problems of Soil Science, Pushchino, Russia
[c]Princeton University, Department of Geosciences, Princeton, New Jersey, USA

ABSTRACT Permafrost microbes may be metabolically active in microscopic layers of liquid brines, even in ancient soil. Metagenomics can help discern whether permafrost microbes show adaptations to this environment. Thirty-three metagenome-assembled genomes (MAGs) were obtained from six depths (3.5 m to 20 m) of freshly cored permafrost from the Siberian Kolyma-Indigirka Lowland region. These soils have been continuously frozen for ~20,000 to 1,000,000 years. Eight of these MAGs were ≥80% complete with <10% contamination and were taxonomically identified as *Aminicenantes*, *Atribacteria*, *Chloroflexi*, and *Actinobacteria* within bacteria and *Thermoprofundales* within archaea. MAGs from these taxa have been obtained previously from nonpermafrost environments and have been suggested to show adaptations to long-term energy starvation, but they have never been explored in ancient permafrost. The permafrost MAGs had greater proportions





Unique genes found in the genomes of permafrost metagenomes

Psychrophiles and cold environments:
microbial life of frozen worlds

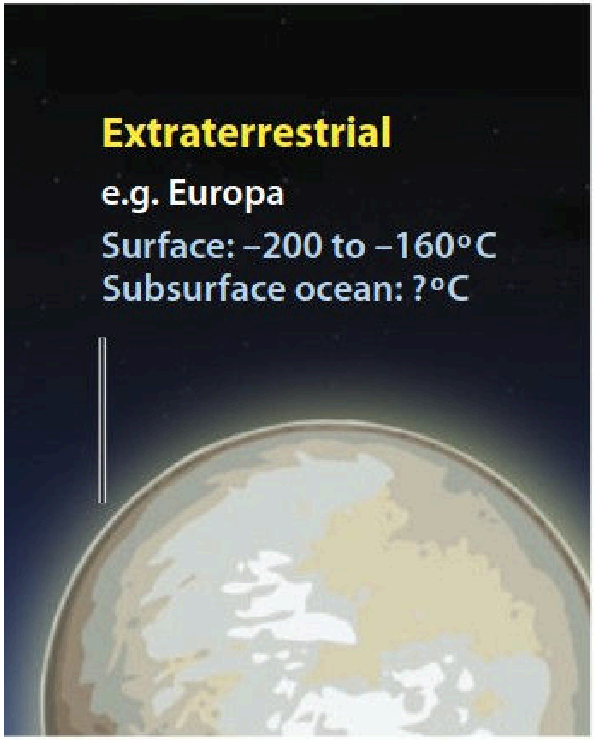
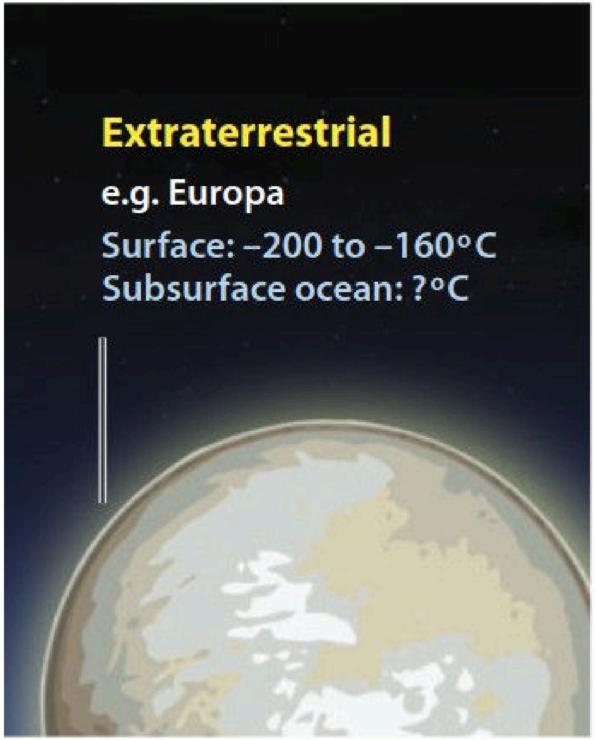


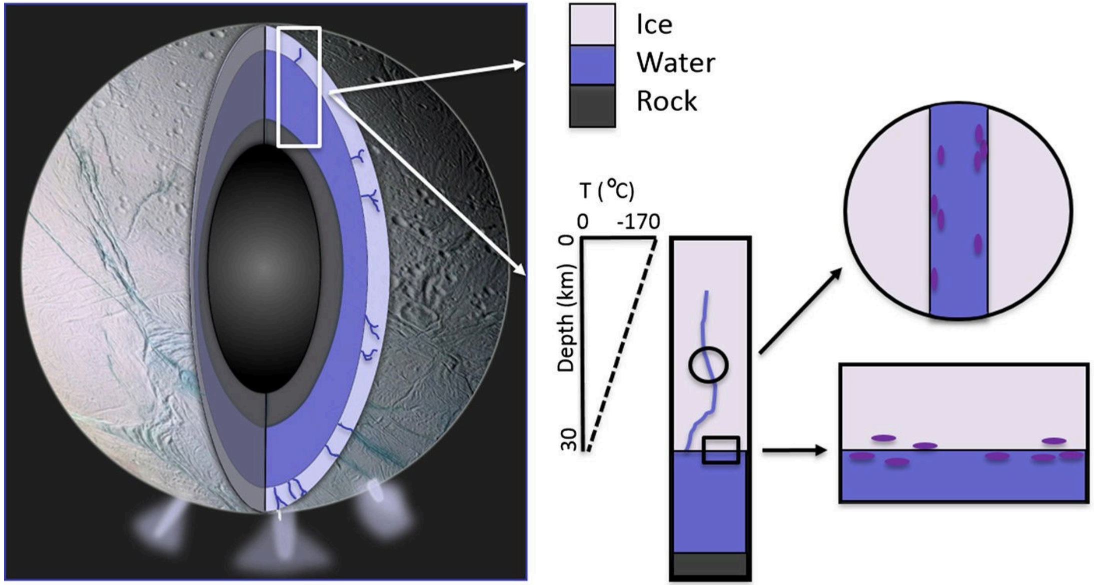
Table 3 Characteristics of some planets and moons from Earth's solar system with the potential to harbor psychrophilic life[a]

Planet/moon	Atmospheric, surface, and subsurface composition	Surface temperature
Earth[b]	O_2, N_2, CO_2 Water exists in all three states (gas, liquid, solid)	−89 to 58°C
Mars	CO_2, N_2 Polar water and CO_2 ice caps	−140 to 20°C
Europa (Jupiter)	O_2 Liquid water ocean may exist under surface ice sheet	−223 to −148°C
Ganymede (Jupiter)	O_2 Water ice	−203 to −121°C
Callisto (Jupiter)	CO_2 (99%), O_2 (1%) Liquid water ocean may exist beneath its surface	−193 to −108°C
Titan (Saturn)	N_2, H_2, CH_4 CH_4 and C_2H_6 exist in all three states as gas, liquid, and solid	−179°C
Enceladus (Saturn)	H_2O, N_2, CO_2, CH_4 Water ice	−240 to −128°C

[a]Data taken from Chown (2011)

Psychrophiles and cold envrioments:
microbial life of frozen worlds

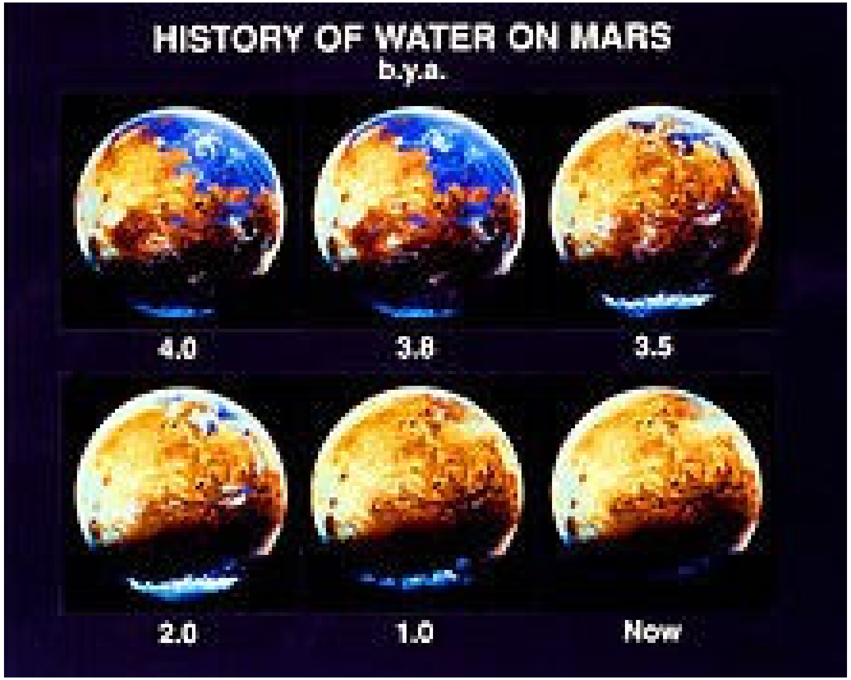


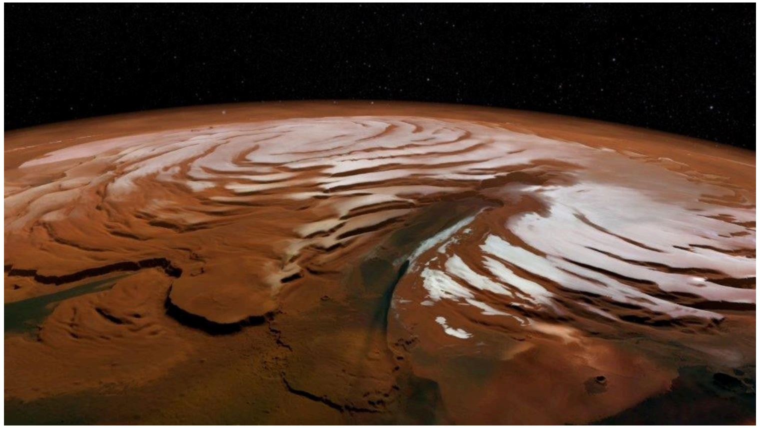


Proposed metabolic pathways for subsurface ecosystems on Europa include methanogenesis, sulfur reduction and iron oxide reduction

Psychrophiles and cold envrioments:
microbial life of frozen worlds

Mars





Mars eventually got cold, and became like the Antarctic dry valleys. Organisms we find in the cold desert regions of Earth are the best models for what life may have been like on Mars early in its history. ”

Suggested reading

Khawar S. Siddiqui, Timothy J. Williams, David Wilkins, Sheree Yau, Michelle A. Allen, Mark V. Brown, Federico M. Lauro, Ricardo Cavicchioli Annual Review of Earth and Planetary Sciences 2013 41:1, 87-115

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Martin, A., & McMinn, A. (2018). Sea ice, extremophiles and life on extra-terrestrial ocean worlds. International Journal of Astrobiology, 17(1), 1-16. doi:10.1017/S1473550416000483

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