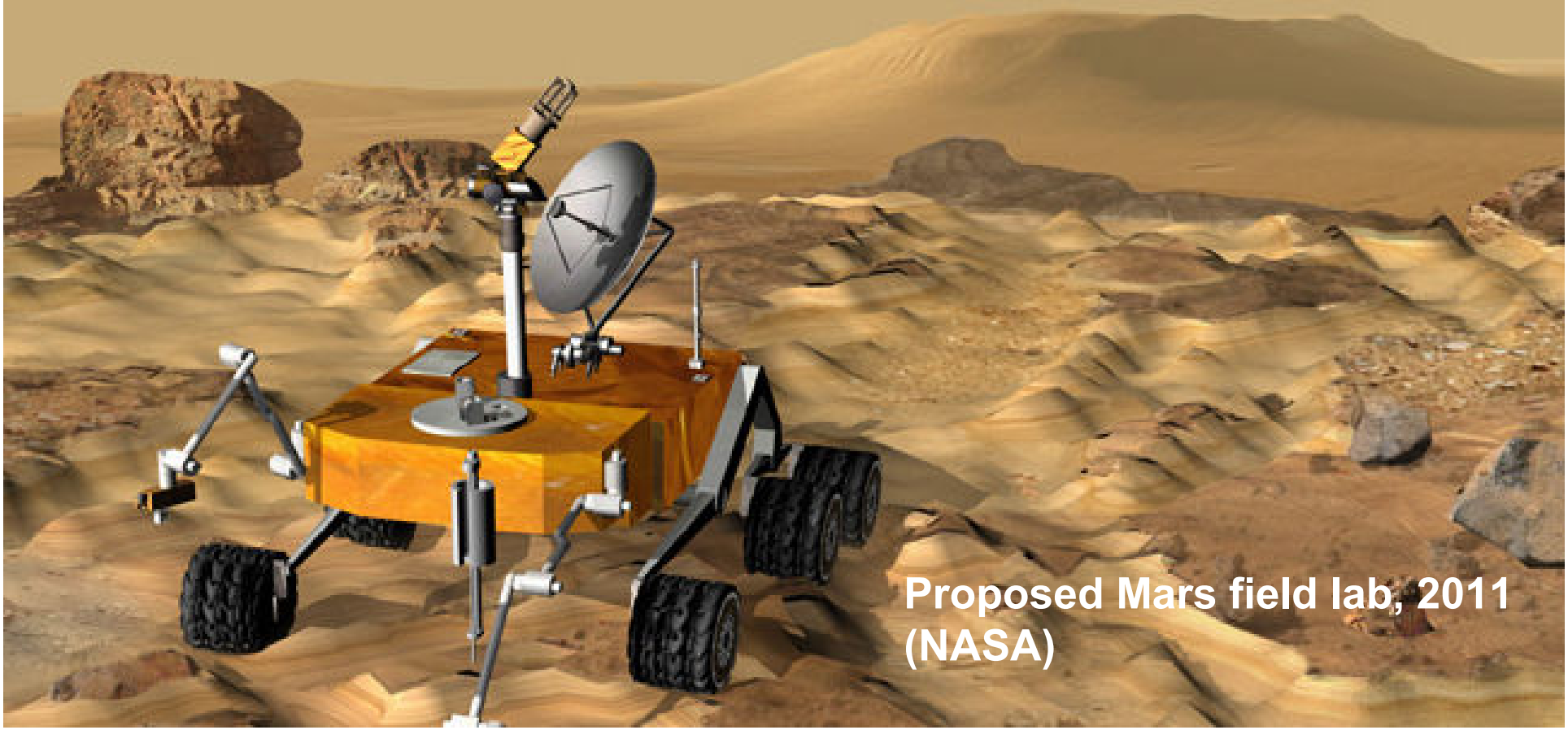
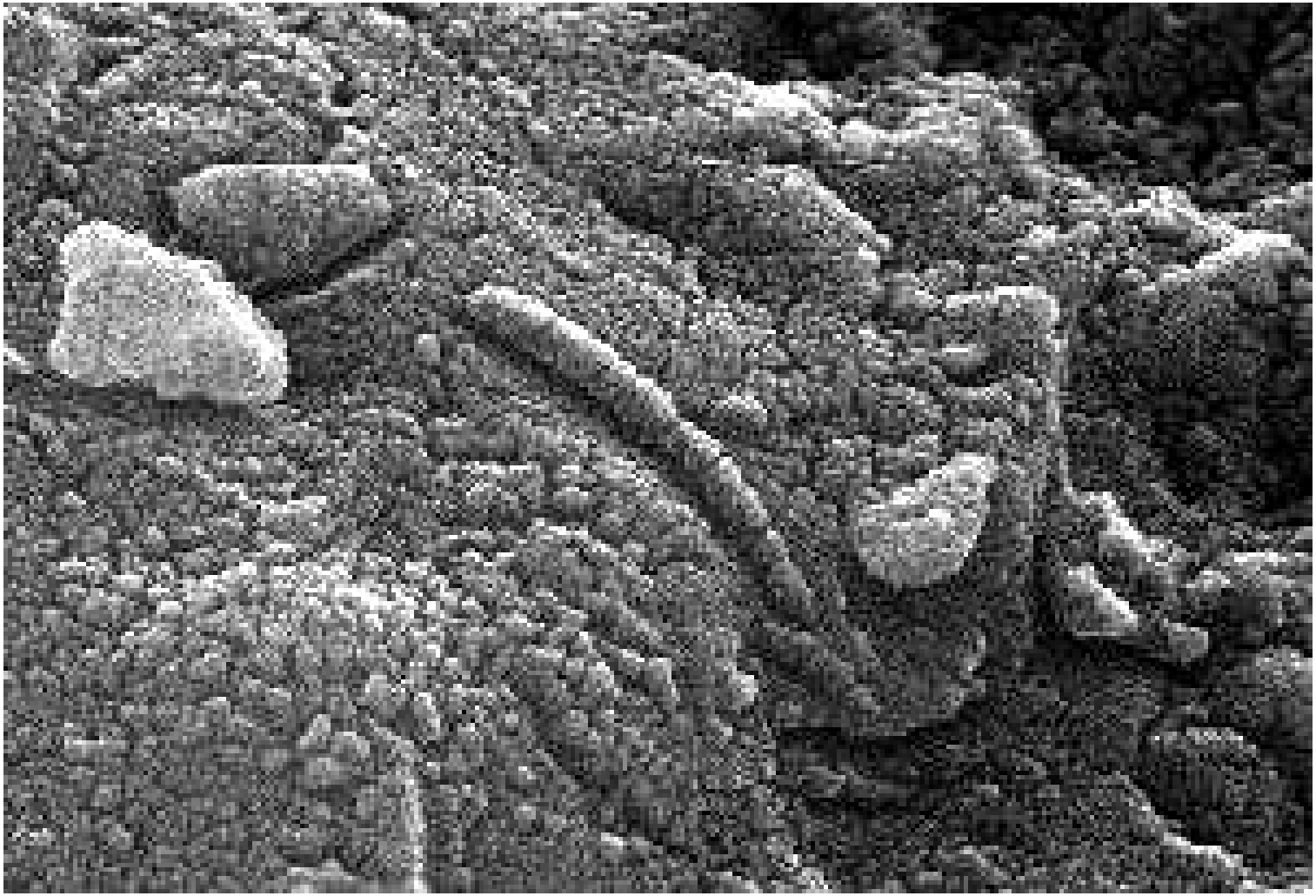
Astrobiology: exploring space (and earth) for life at the extremes

Guest lecture by Virginia Walker
Biology, Queen's University



Proposed Mars field lab, 2011 (NASA)

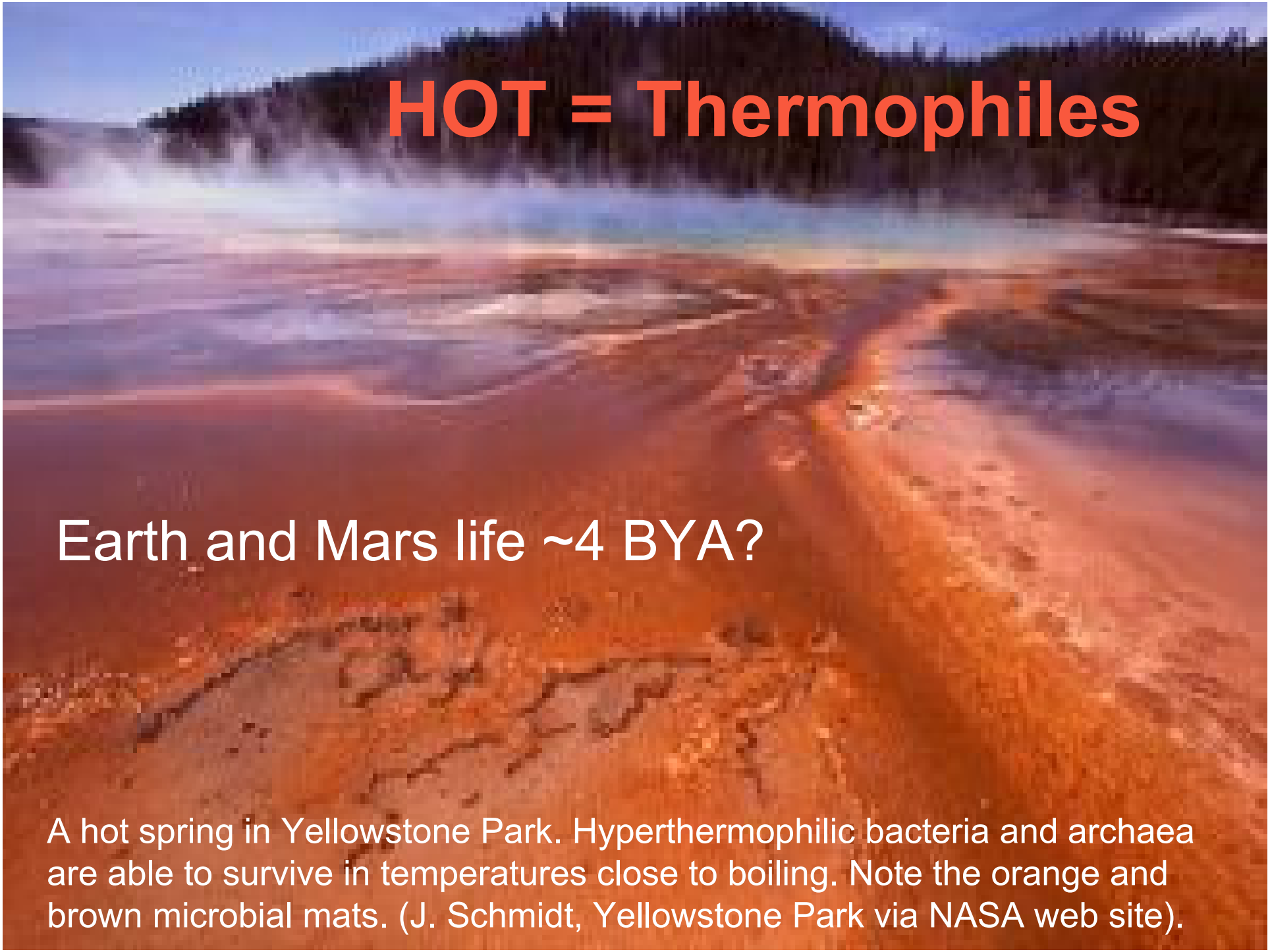


Biogenic-looking features in ALH84001 Martian meteorite. NASA.

Astrobiology or exobiology- how can we study it when we aren't 'there' yet?

NASA: Three of NASA's 7 astrobiology goals are to understand life on this earth: its past, its future and the environmental limits to life

Commander Spock: "That is the exploration that awaits you! Not mapping stars and studying nebula, but charting the unknown possibilities of existence"



A hot spring in Yellowstone Park. Hyperthermophilic bacteria and archaea are able to survive in temperatures close to boiling. Note the orange and brown microbial mats. (J. Schmidt, Yellowstone Park via NASA web site).

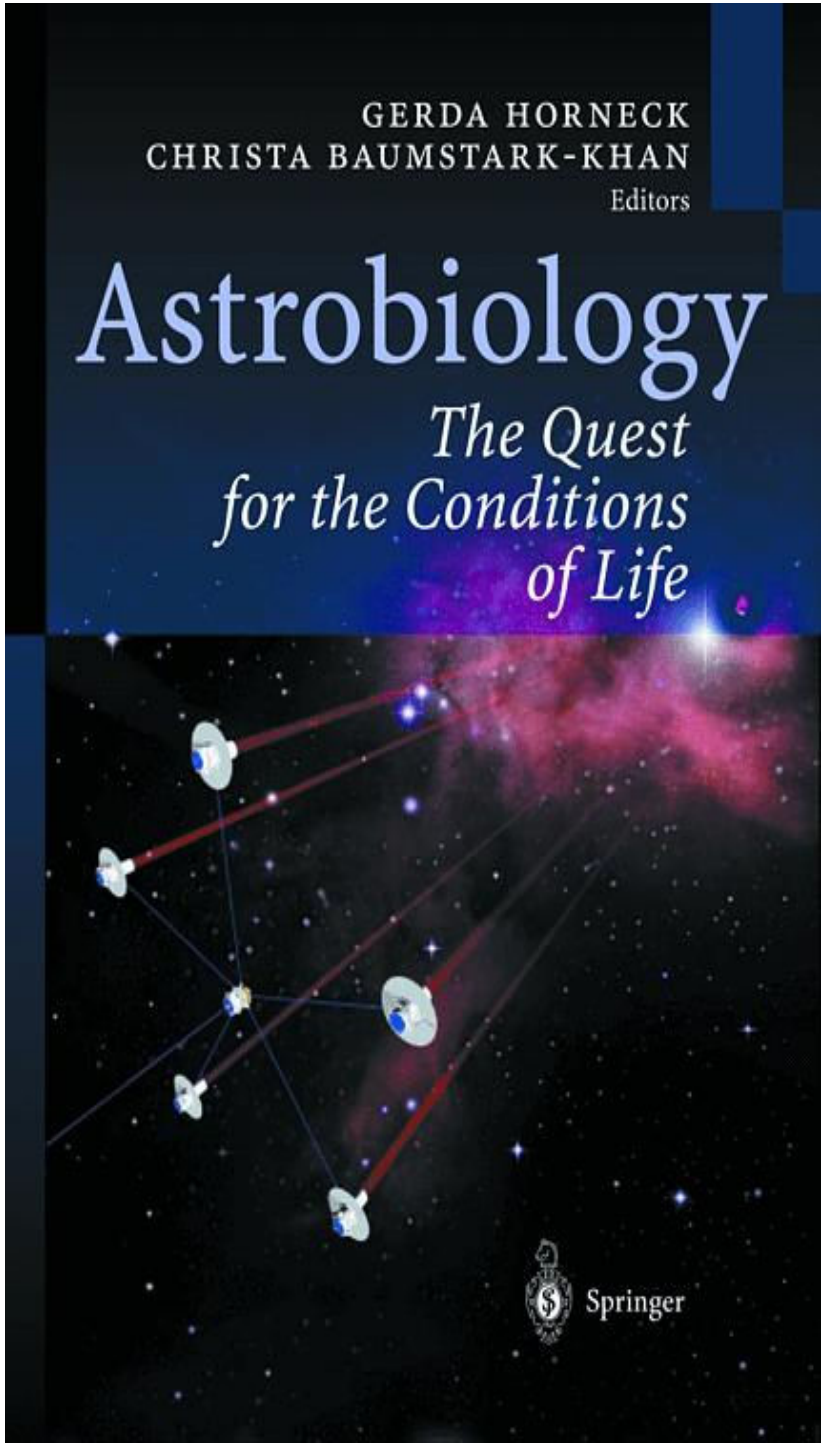
In 2005 it was reported that photosynthetic bacteria had been found at the edges of a thermal vent (350ºC) and in the deep ocean (2ºC; 2.4 km; Pacific Ocean), just off Mexico. *

* PNAS 102:9306 Photo courtesy of team leader: Dr. J.T. Beatty, UBC

It is speculated that hot vents (as well as the volcanoes seen by Galileo) might also be found on Io, a moon of Jupiter.

Other Extremophiles:

1. Pressure-tolerant microbes (expeditions to the Mariana trench)
2. Acidophiles (pH 1-5) found in mine tailings, sulfur pools and our own stomachs
3. Alkaliphilic microbes found in alkali and salt lakes
4. Halophilic microbes (>1.5 M) from places like Great Salt Lake and the Dead Sea may also be UV and desiccation resistant and survive without oxygen.
5. Xerophiles survive with little water (found in deserts).
6. Psychrophilic microbes grow at temperatures close to 0ºC.



So microbes can grow at temperatures close to 0ºC, but many microbes on earth are killed by subzero temperatures- this is the basis of the sewage bulbs in polar regions, as well as Delta Engineering's Snowfluent® waste water treatment

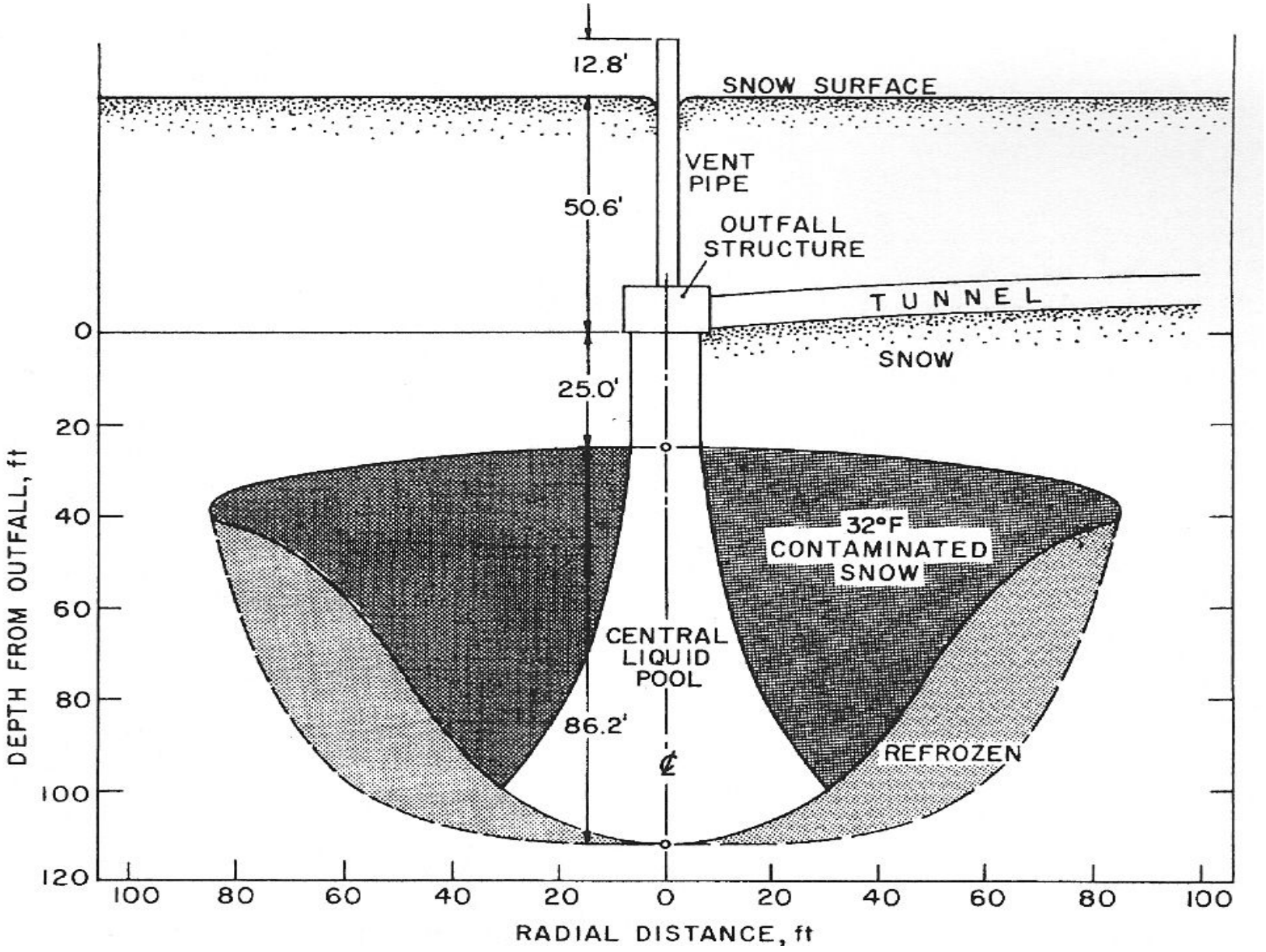
Sewage being turned into snow at the Swift Current and the Westport facilities

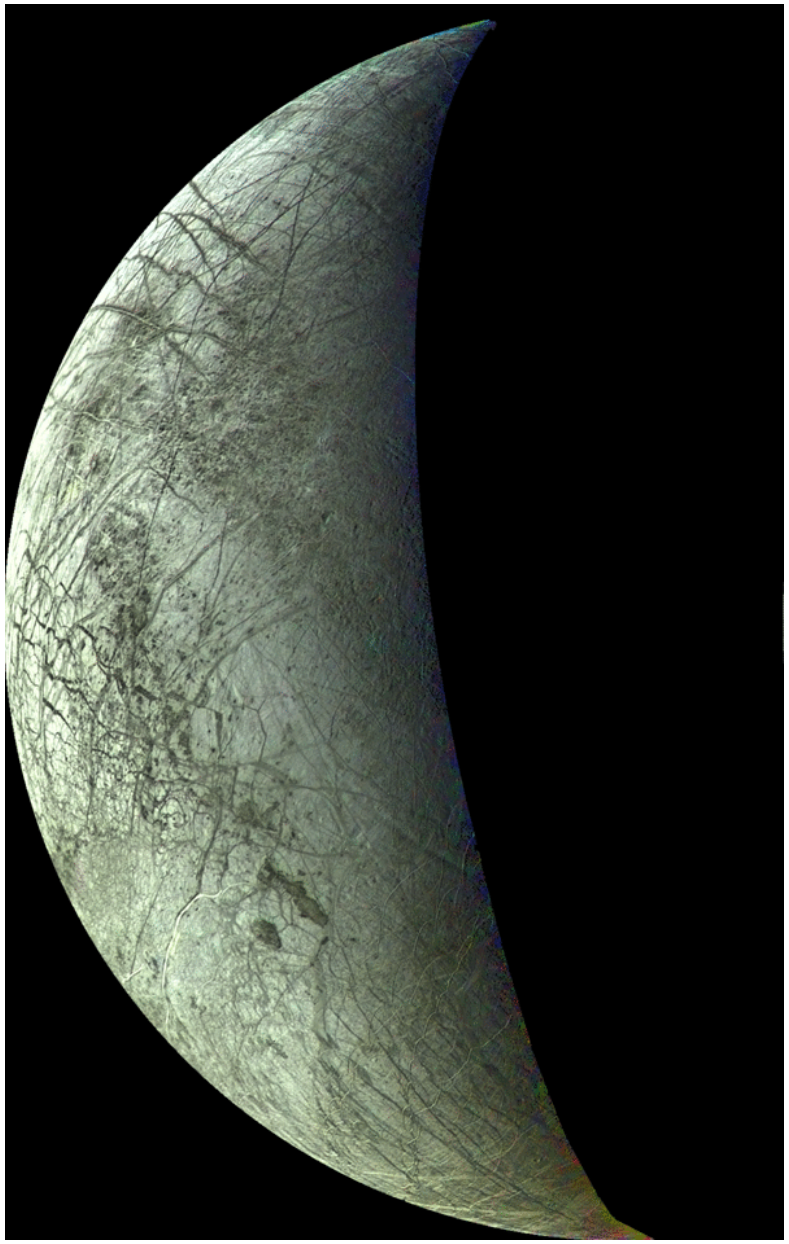
Slurry hits the slopes

With new "Snowfluent" process — freezing manure into snow — skiers could be sloloming on slurry

Canada's harsh climate gets blamed for higher building, energy and feed costs. But Alberta pork producers are betting our chilly weather is the key to a new treatment for liquid manure.

Sewage bulbs in polar regions work by freezing the sludge. Most microbes, including pathogens, probably die.

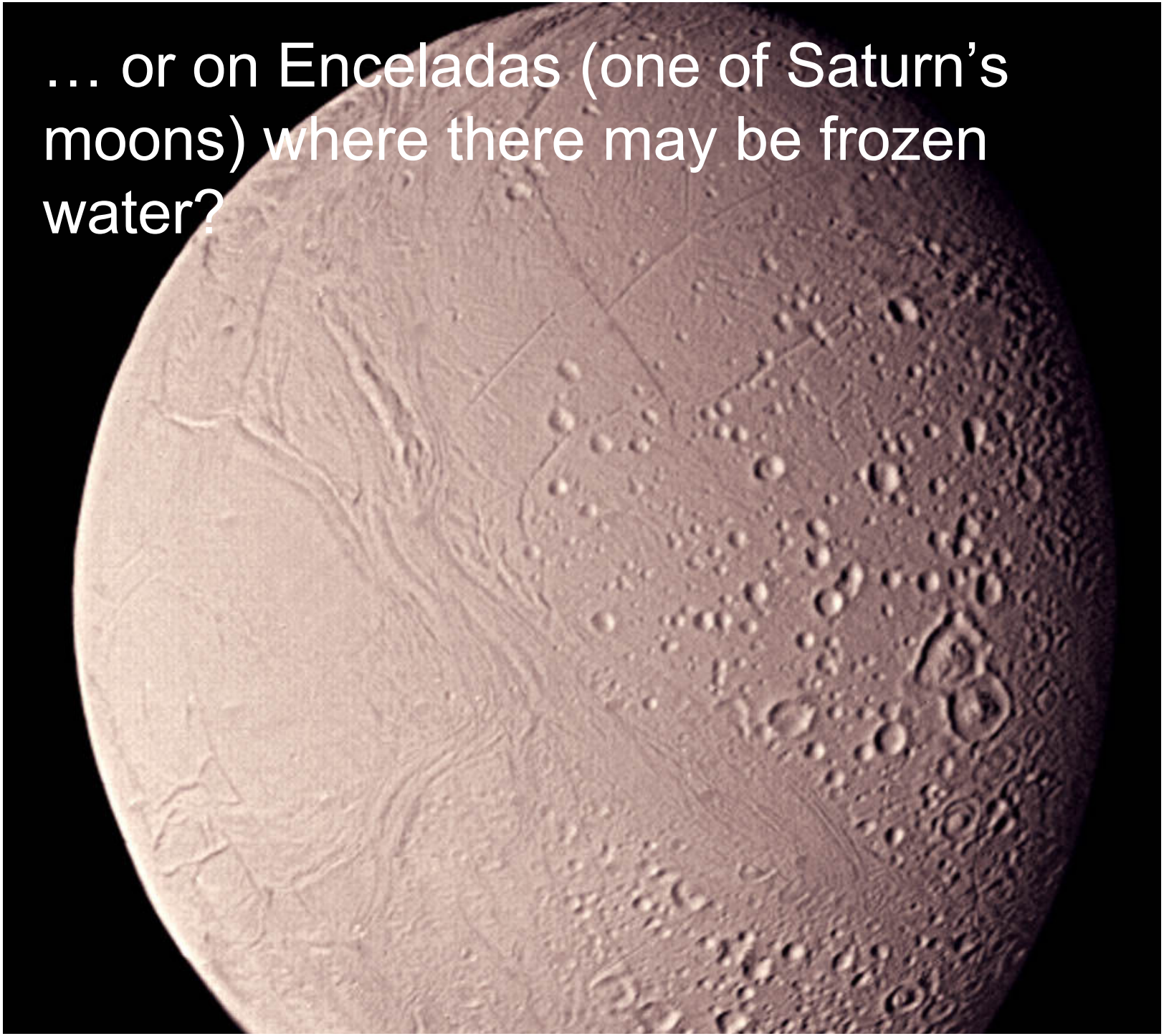




Europa appears to be covered by a water-ice shell $\leq$ 150 kms thick. Given the experience here on earth, is it unlikely that life exists here?

http://www.lpi.usra.edu/resources/outerp/euro.html

… or on Enceladas (one of Saturn's moons) where there may be frozen water?



http://www.solarviews.com/raw/sat/enceladx.jpg

http://www.damocles-eu.org/artman/uploads/arctic_globe-px.jpg

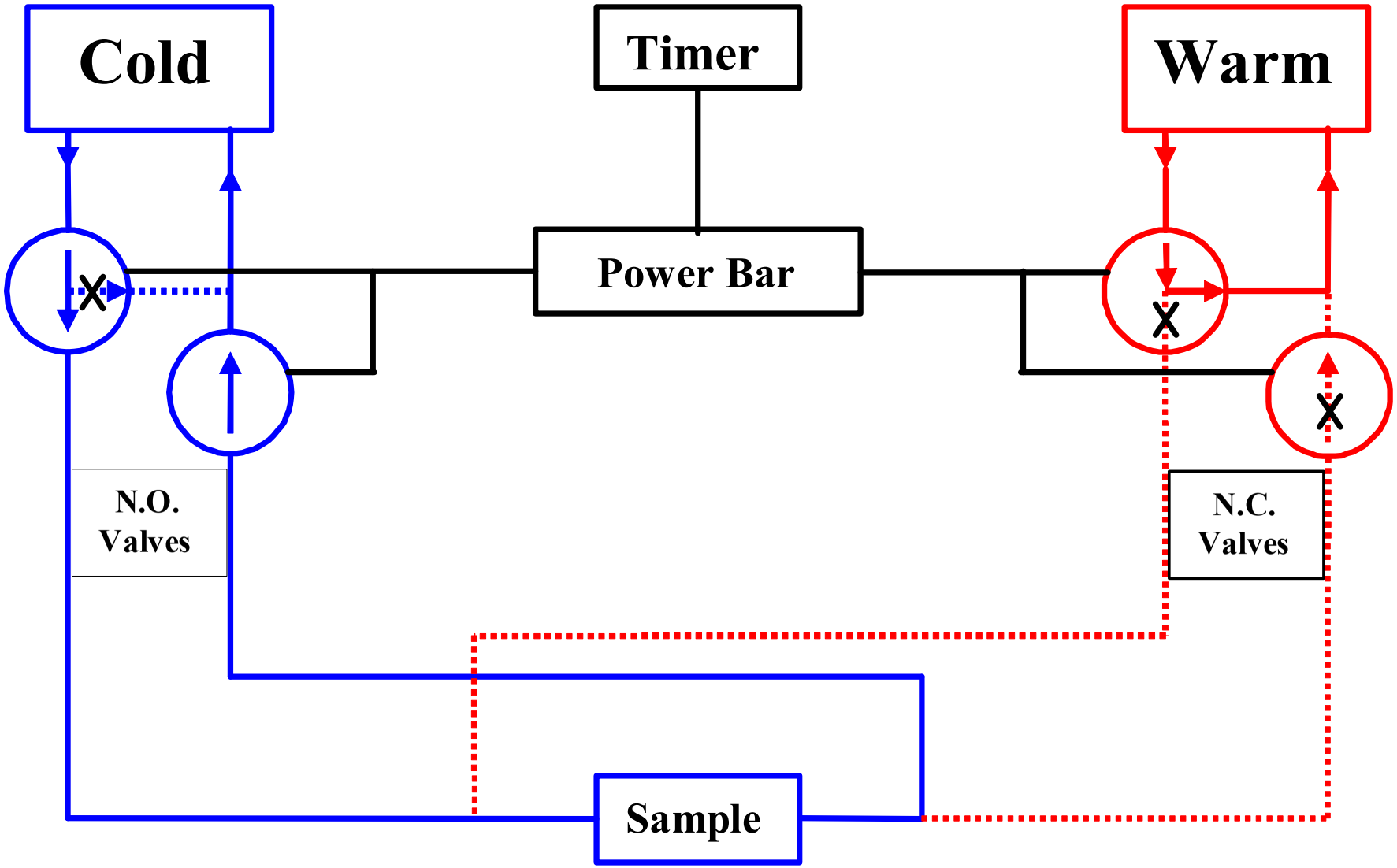
Finding out: Expeditions to the Antarctic in search of psychrophilic microbes and their secrets of survival

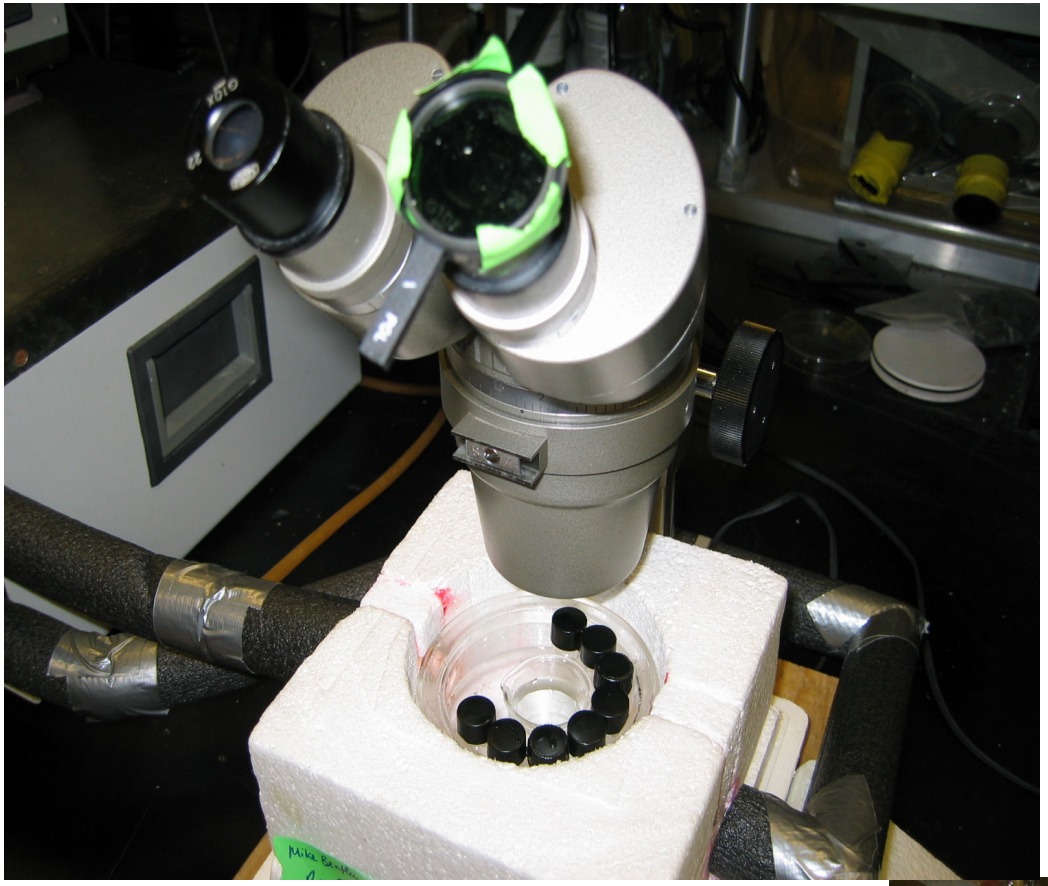
Why not avoid the expense and the bureaucracy and just dig a little soil from your own backyard?

Methods: Multiple soil samples were taken from one location and composted prior to use, then added to dilute nutrient medium for culture

1. Selection for freeze-thaw resistant microbes

Cryocycler diagram



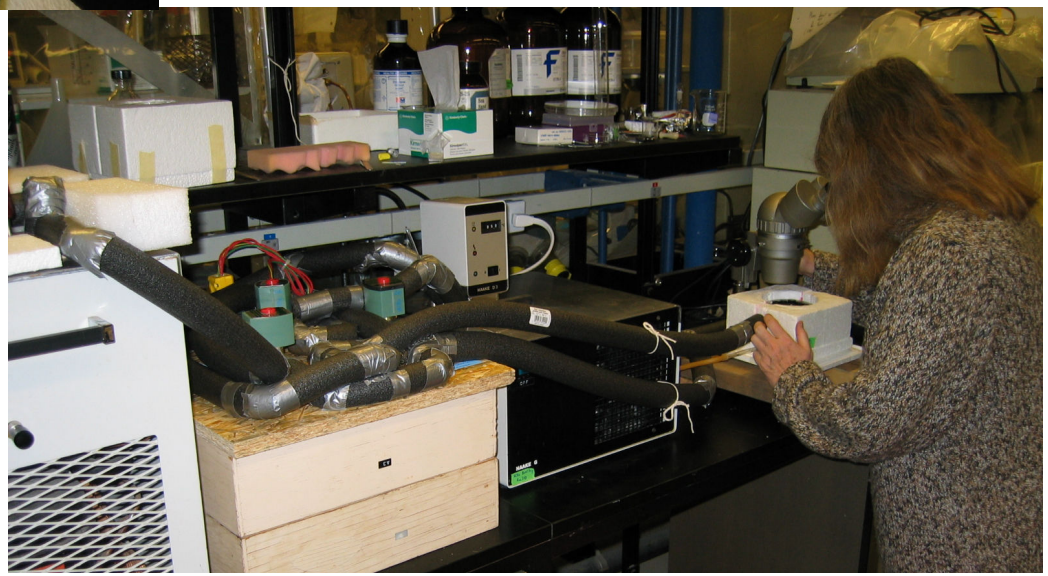


<u>Testing for survival</u>:

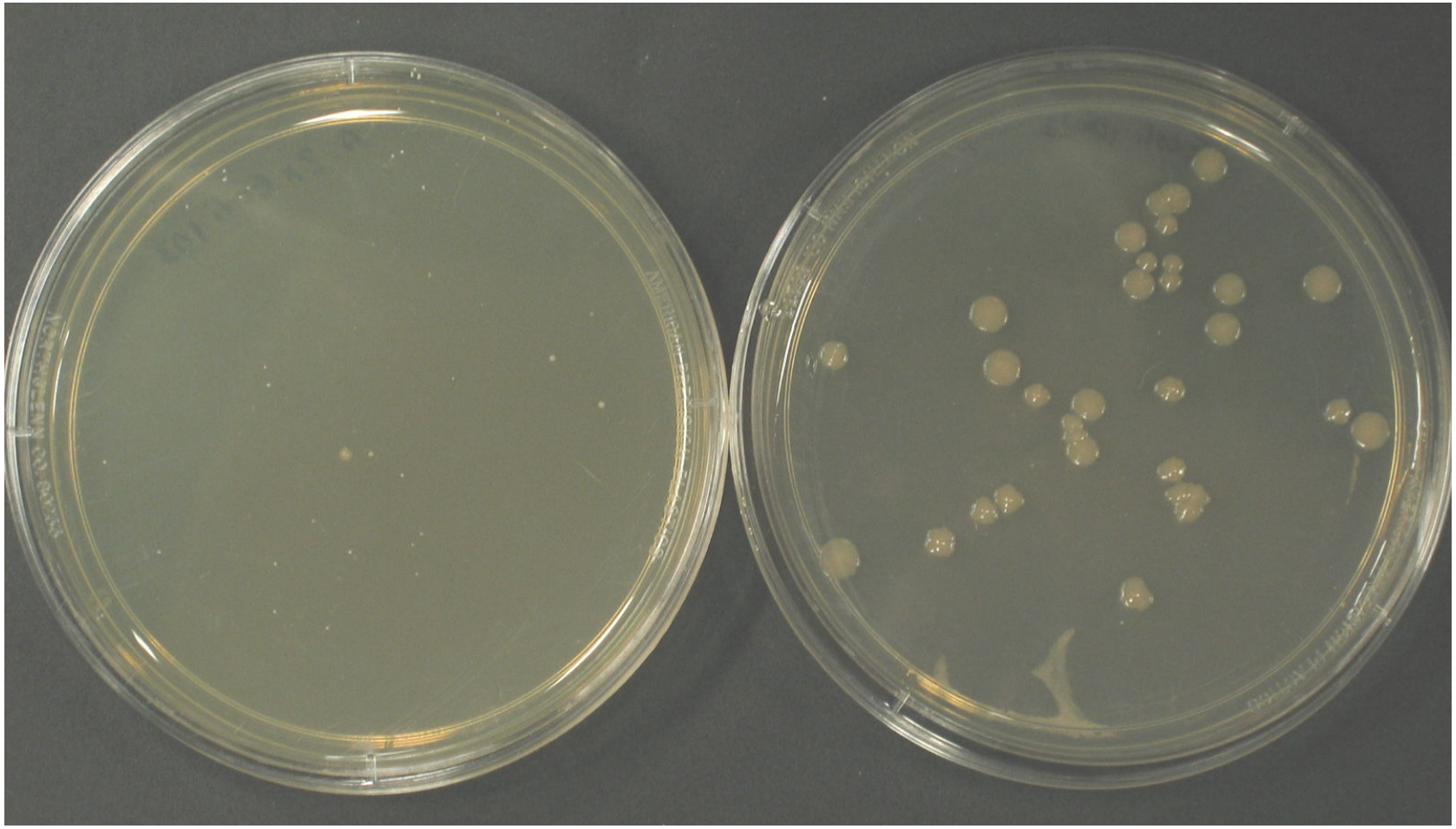
1. *E. coli*- freeze sensitive?

2. *Pseudomonas*- relatively freeze resistant?

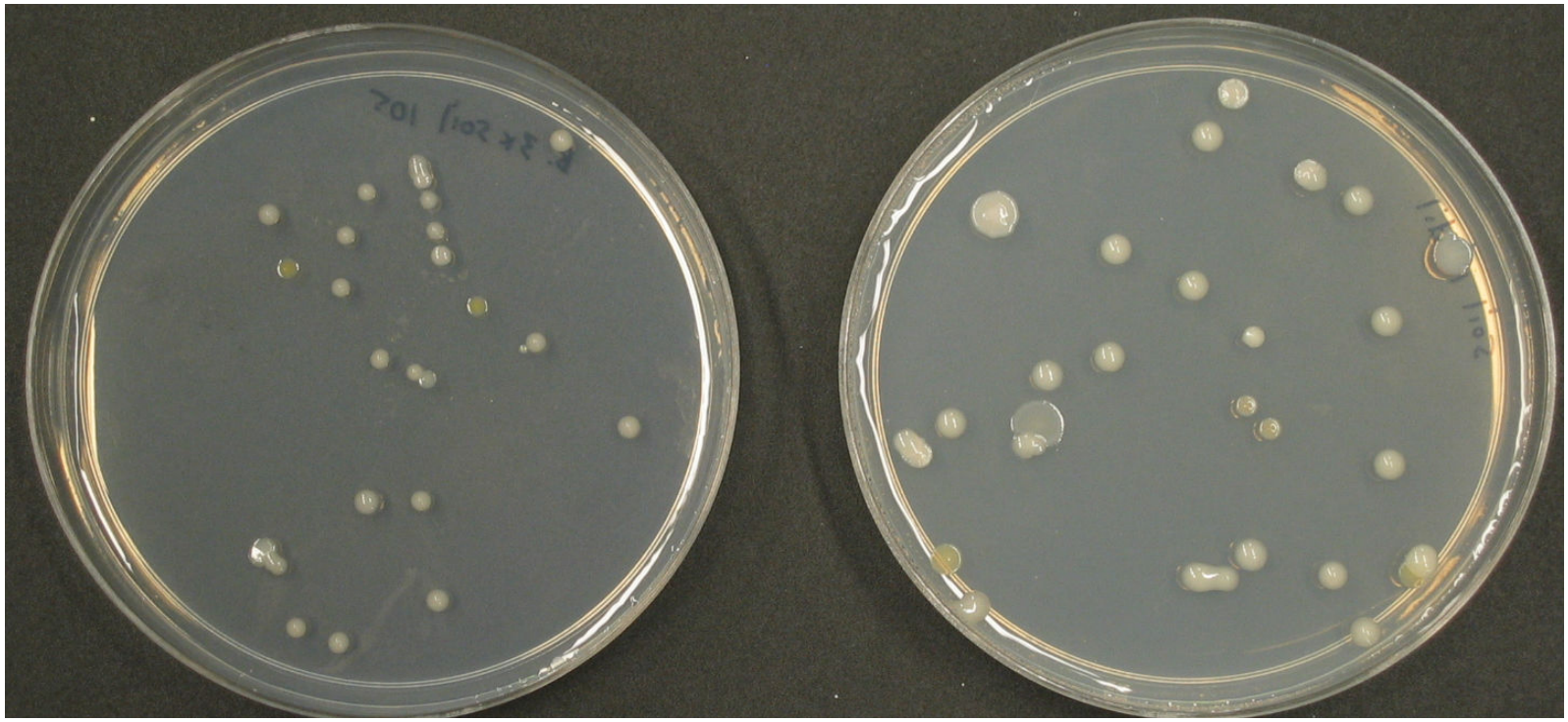
3. Calgary soil bacteria- unknown



E. coli plated directly from culture (right) or subjected to 3 free-thaw cycles (left) and allowed to grow for ~30 h.

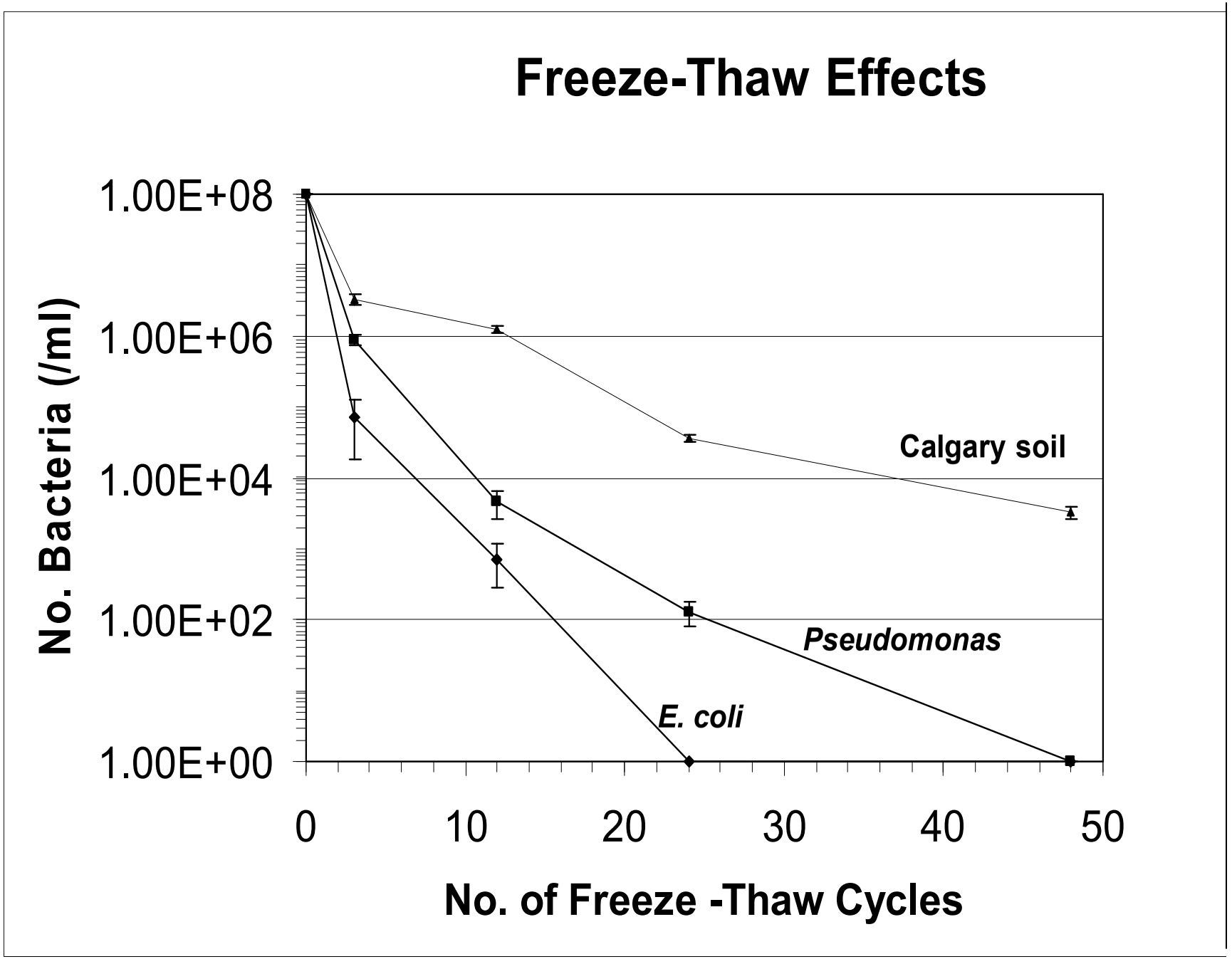


Calgary soil bacteria plated directly from culture (right) or subjected to 3 free-thaw cycles (left), after ~30 h

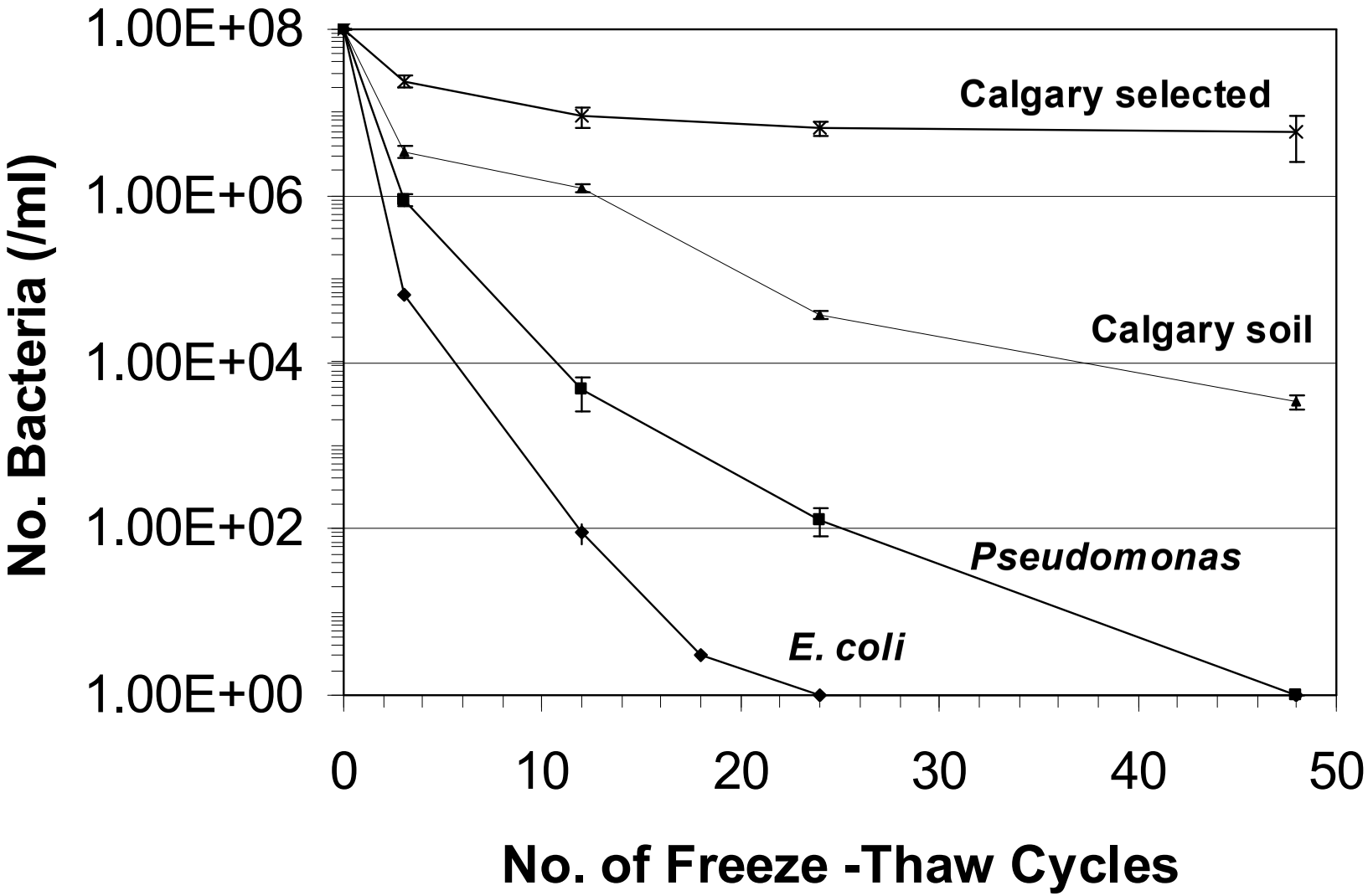


After 3 freeze-thaws: *E. coli* colony size is **36%**, *P. chloroaphis* colony size is **50%** and Calgary soil colony size is **70%**, compared to controls.

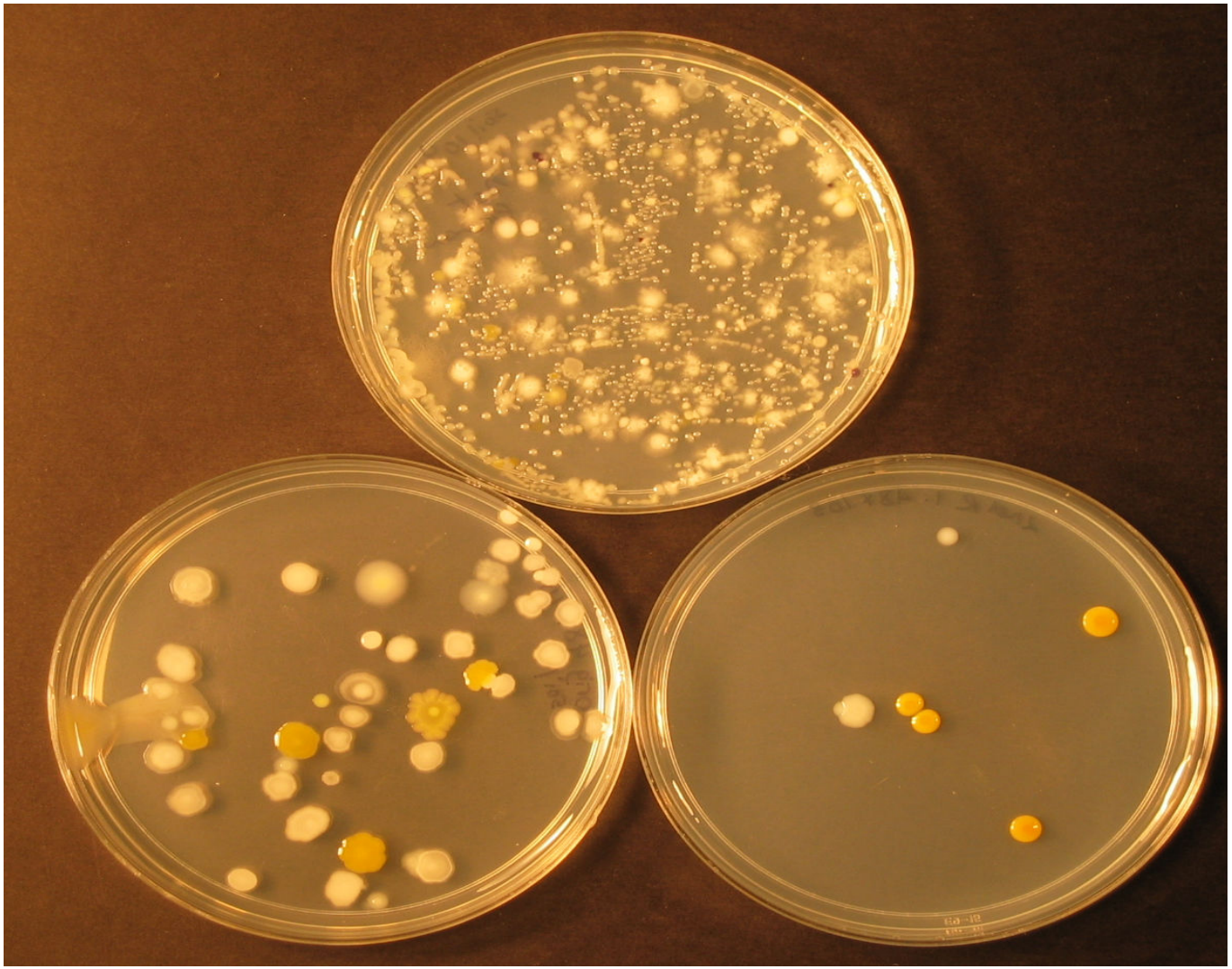
*Thanks to Brendan for first noticing that previously frozen bacteria have a lag period


Freeze-Thaw Effects
No. Bacteria (/ml)
1.00E+08
1.00E+06
1.00E+04
1.00E+02
1.00E+00
0
10
20
30
40
50
No. of Freeze -Thaw Cycles
Calgary soil
Pseudomonas
E. coli

Viability of bacterial cultures after serial freeze-thaw treatments in the cryocycler

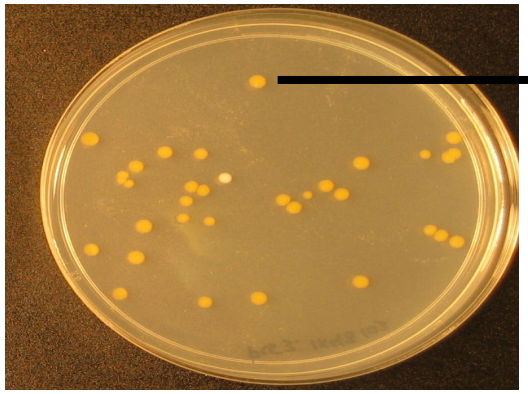


The complexity of the population dramatically decreases as the number of freeze-thaw treatments increases



** original, 48 freeze-thaw, and 2 x 48 freeze-thaw treatments*

Identification of the selected "Chinook" bacteria

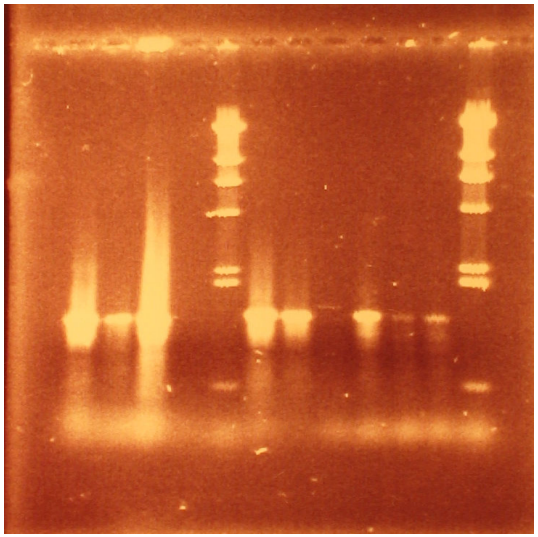


Pick a single colony, streak and isolate a progeny colony.
Use this colony to inoculate liquid medium.

Freeze-thaw/ lysozyme/ 95°C

DNA isolation

PCR with rDNA primers

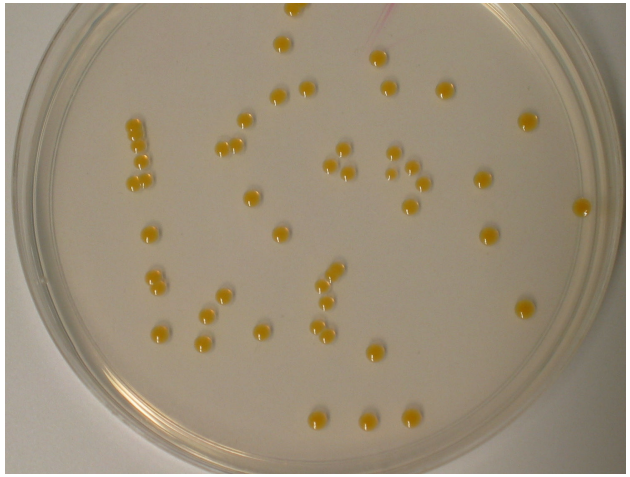


DNA sequence

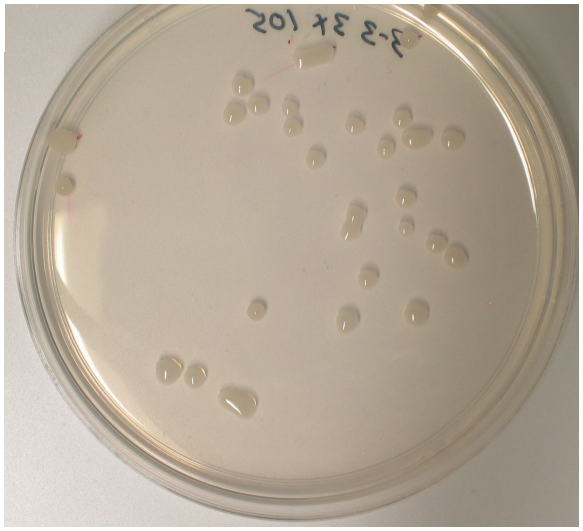
Genbank data base search

Some of the freeze-thaw tolerant Chinook strain isolates

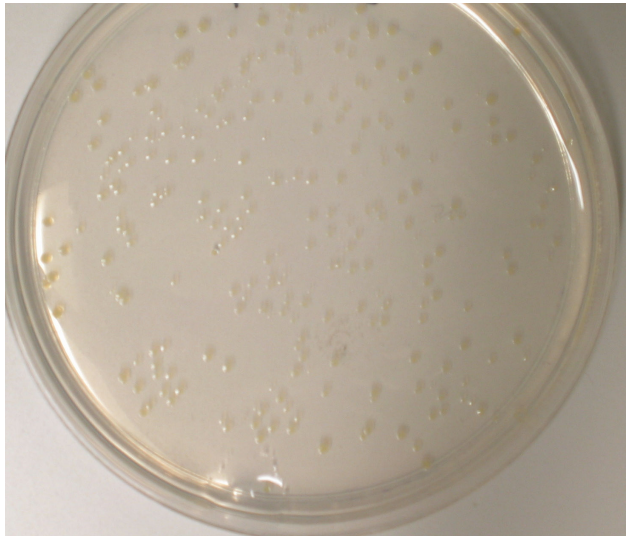
Chryseobacterium (98%; Antarctic glaciers)



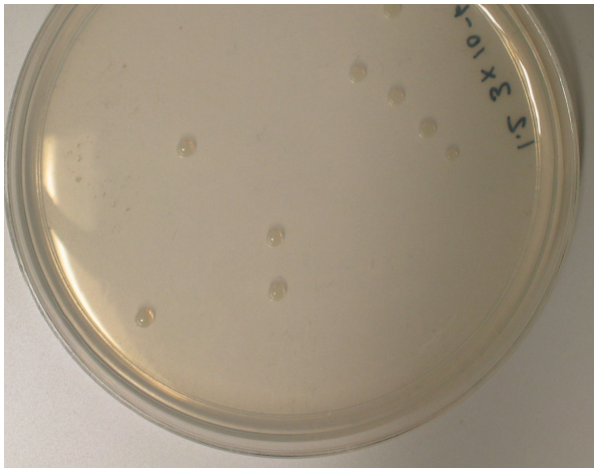
Acinetobacter (99%; in glaciers)



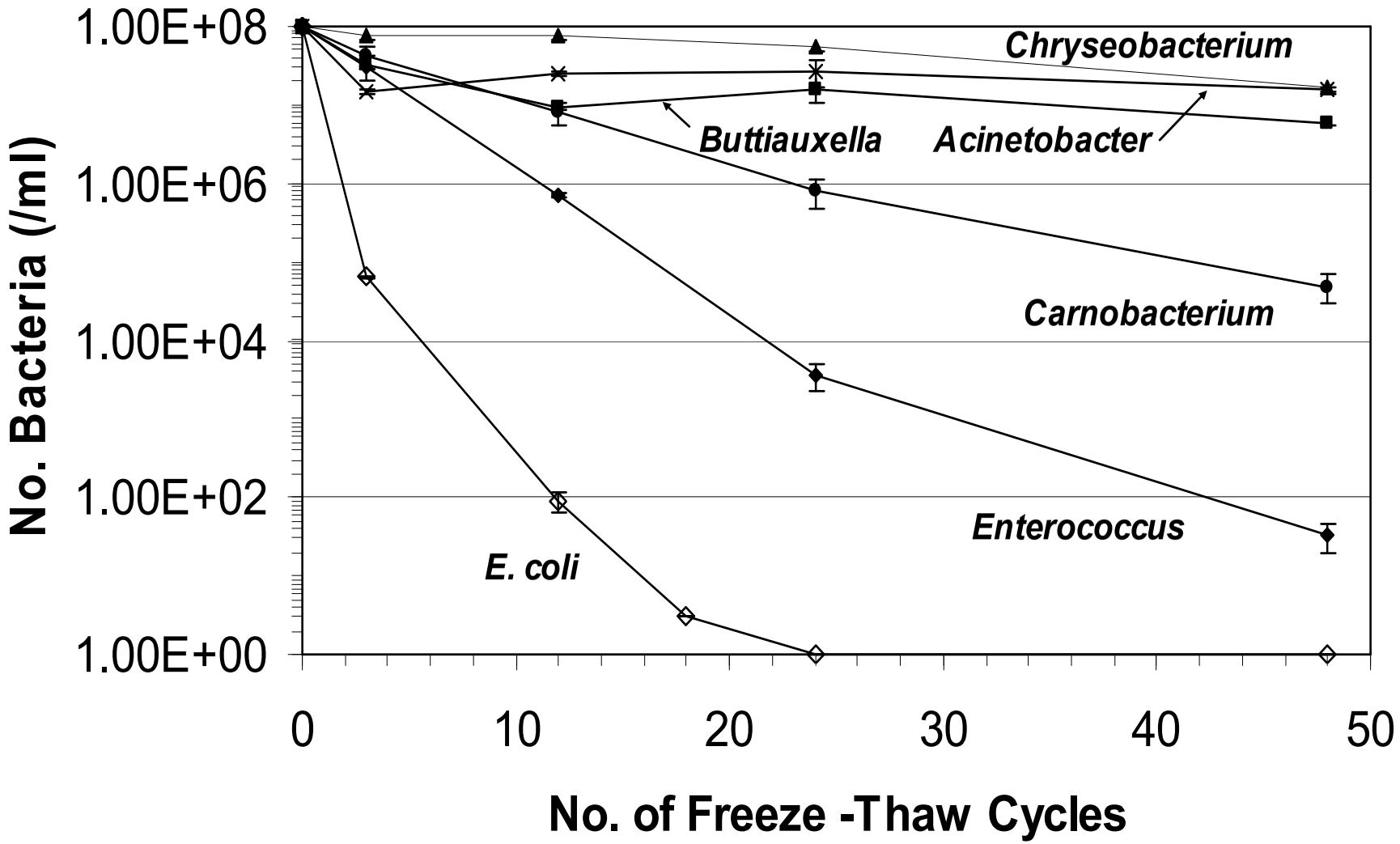
Enterococcus (99%)



Buttiauxella (98%; known psychrophile)



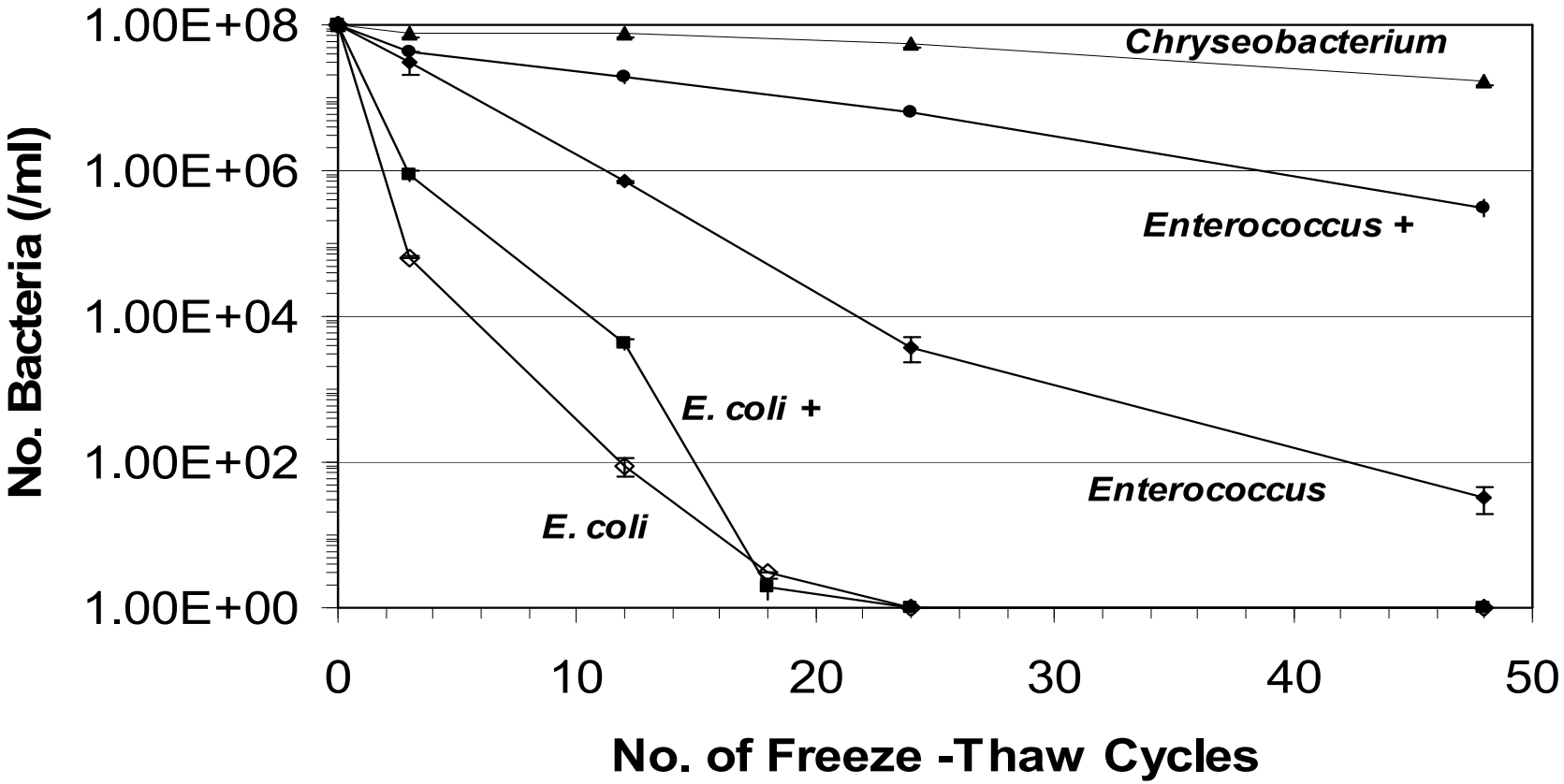
Viability of Chinook isolates after serial freeze-thaw treatments



Tested: ***Enterococcus*** : cells & medium; ***E. coli*** : cells & medium ***Chryseobacterium*** : cells & medium

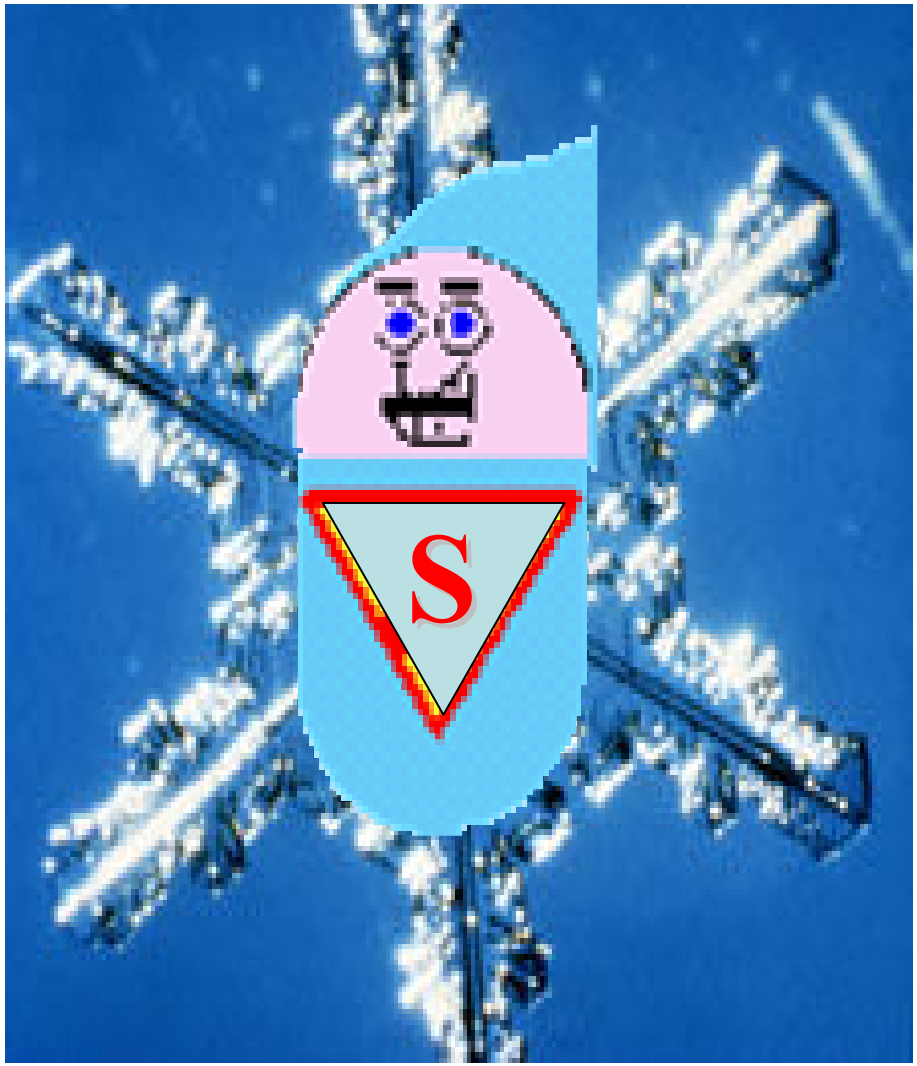
Enterococcus + : cells & *Chryseobacterium* medium
E. coli + : cells & *Chryseobacterium* medium

Freeze-thaw resistance of "reconstituted" Chinook strains



Why are these Chinook strains so resistant to freeze-thaw conditions?

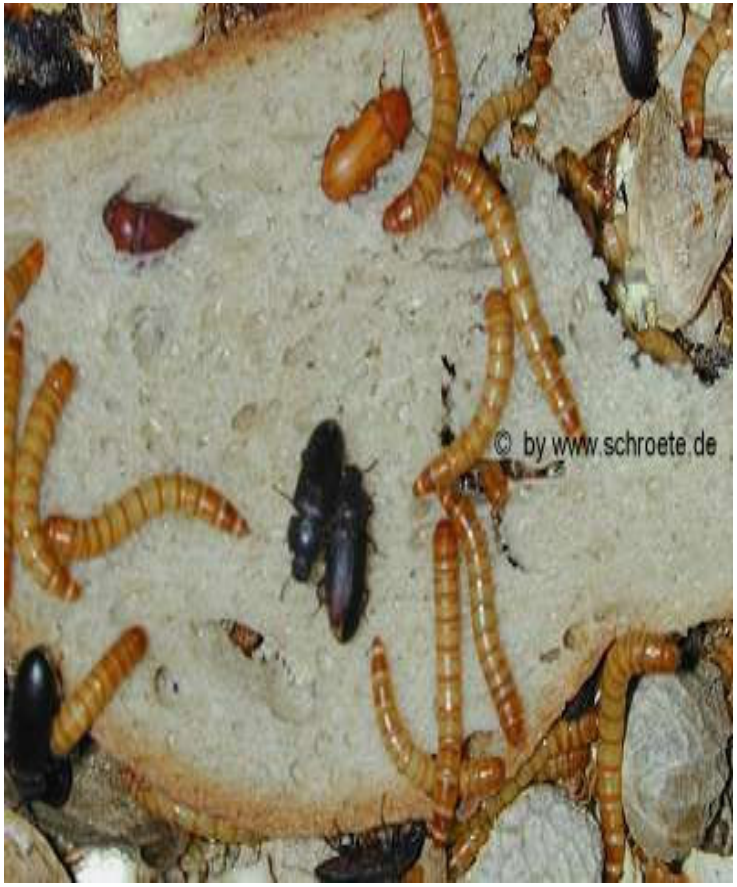
Hypothesis:
Maybe they have proteins, like antifreeze proteins (AFPs) that protect them from freezing damage and other microbes (like *E. coli*) do not have these proteins.



AFPs have been found in some fish like the ocean pout, *Macrozoarces americanus*

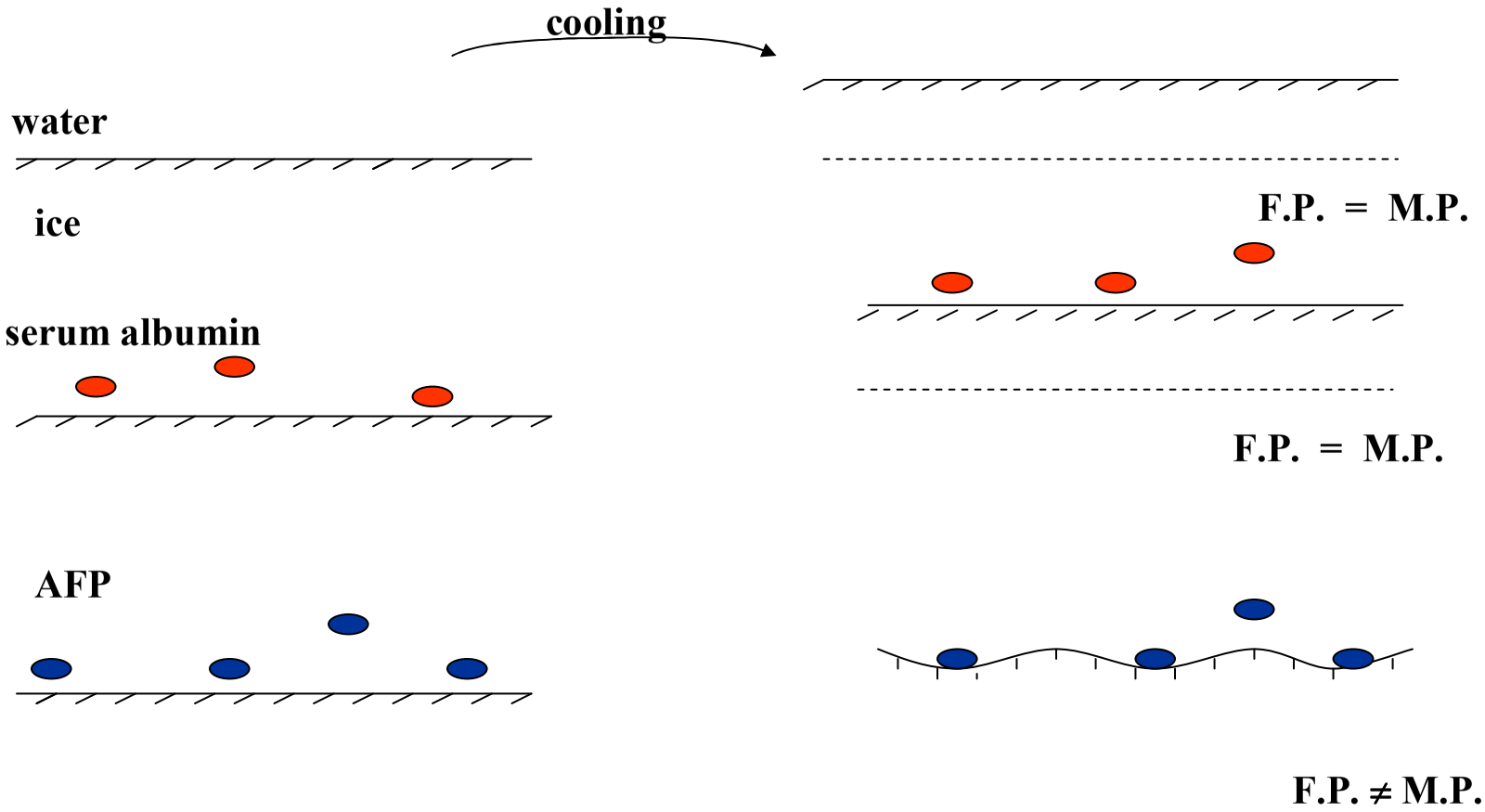
AFPs have also been found in insects

Choristoneura fumiferana
spruce budworm



Tenebrio molitor
yellow mealworm

Antifreeze proteins adsorb to ice

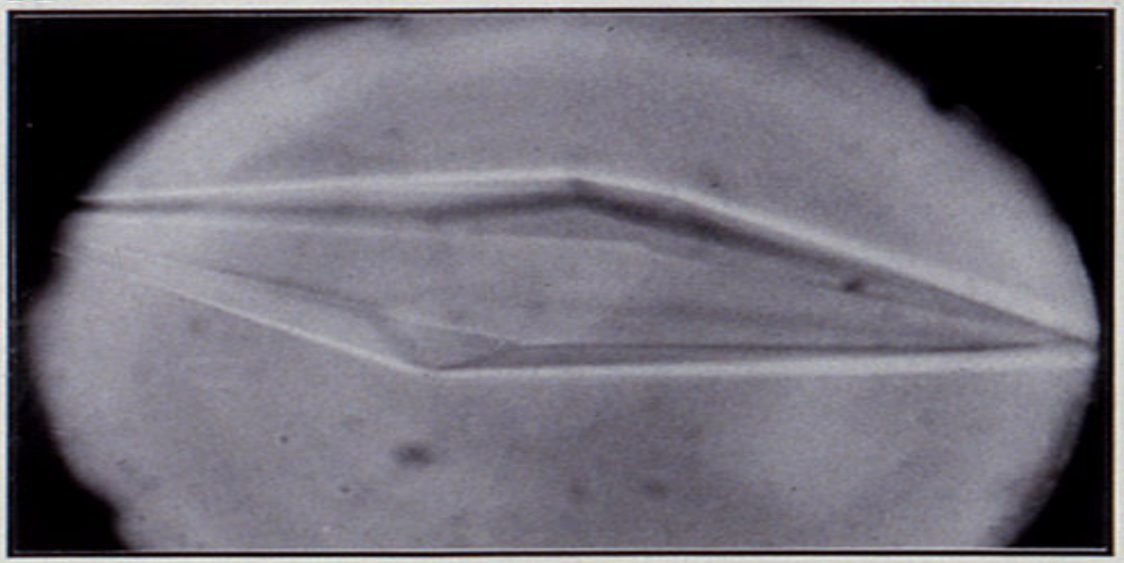


Adsorption-inhibition mechanism for inhibiting ice growth

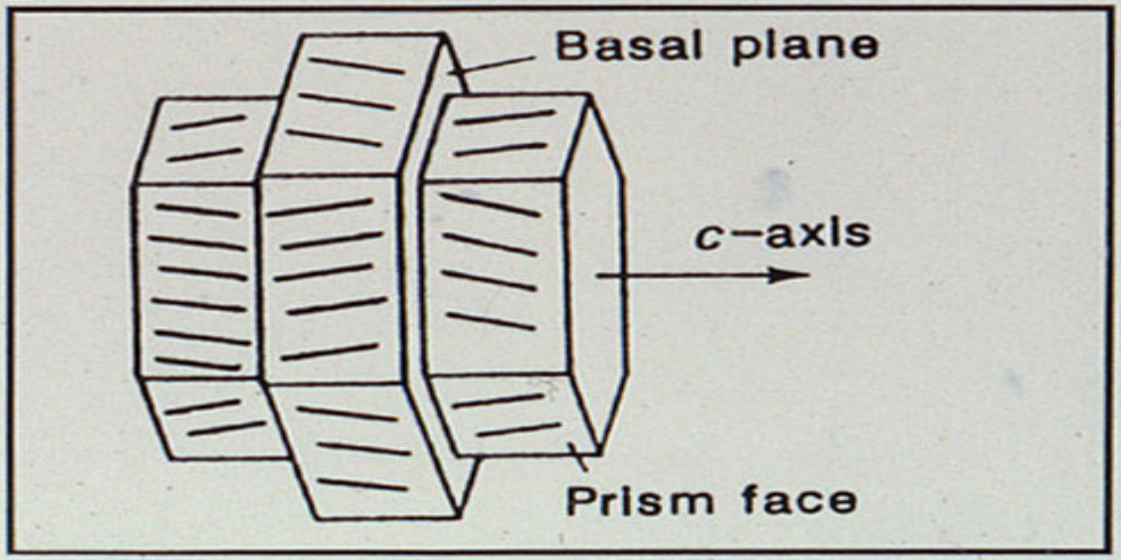
Thermal hysteresis = difference between freezing point (F.P.) and melting point (M.P.) (^{o}C)

ICE CRYSTALS IN AFP SOLUTIONS

A



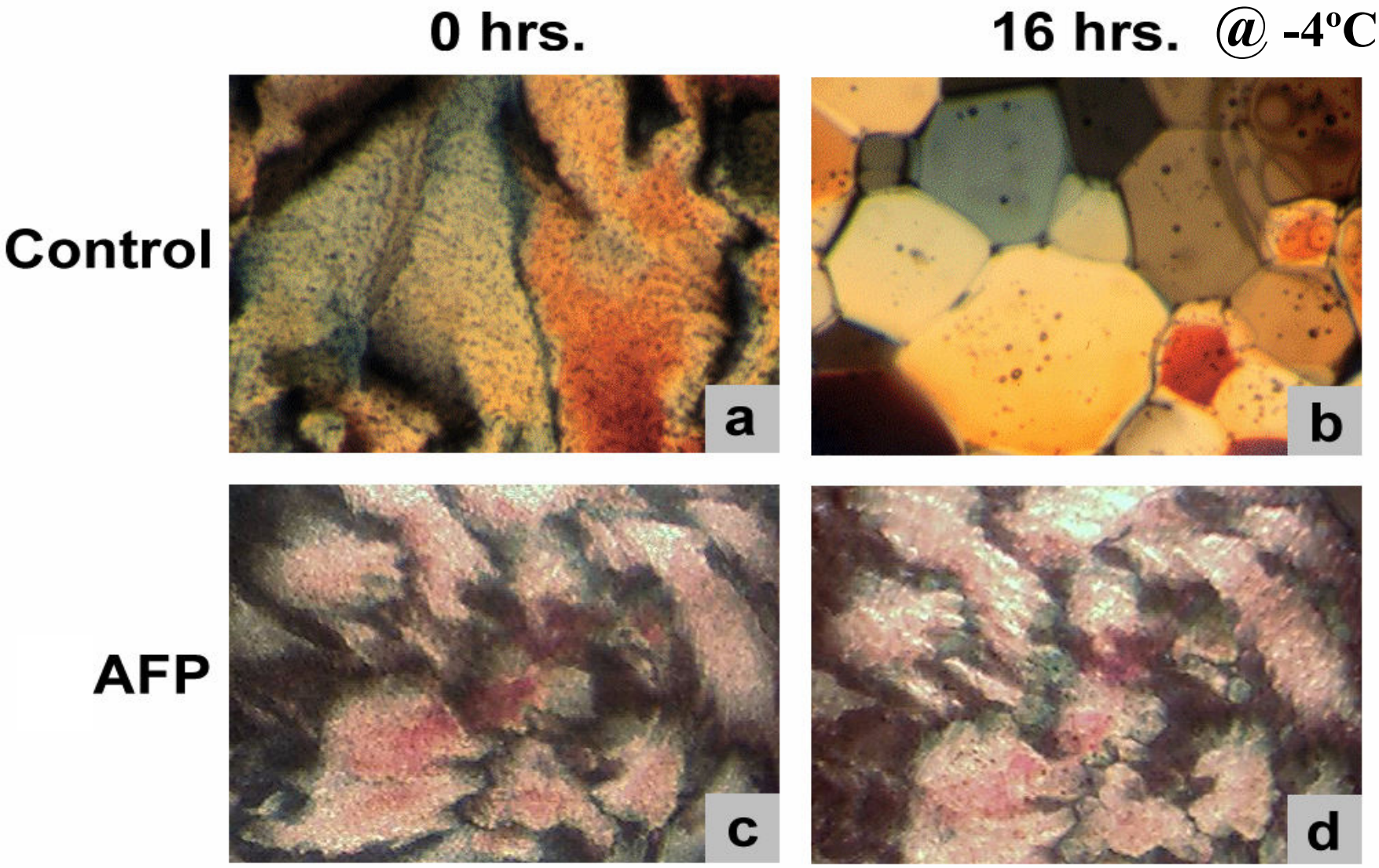
B



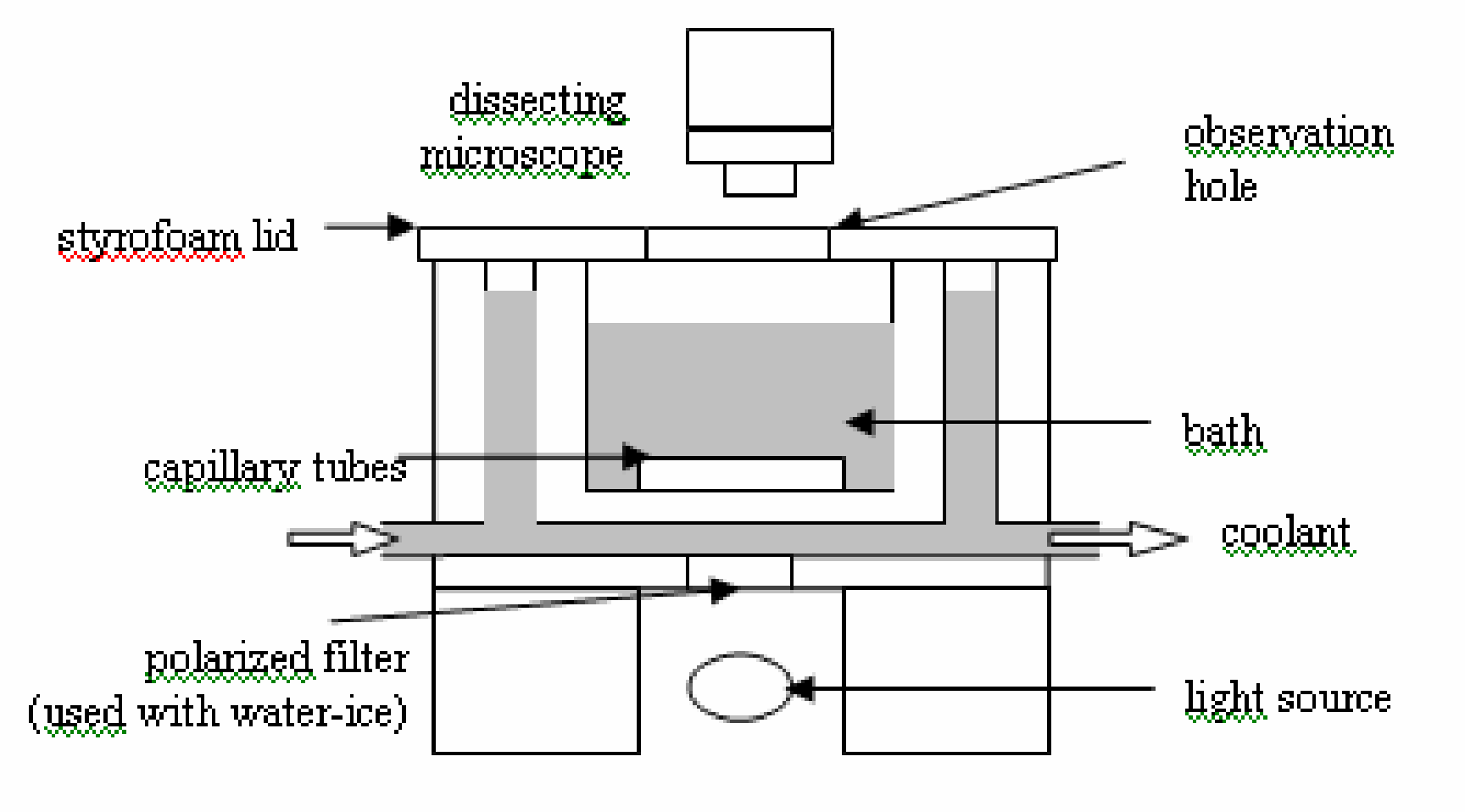
But, if these fish or insects freeze, they die. The selected Chinook strains freeze and don't die.

Could the microbes still have AFPs?

Another property of AFPs is that they can inhibit ice recrystallization……..

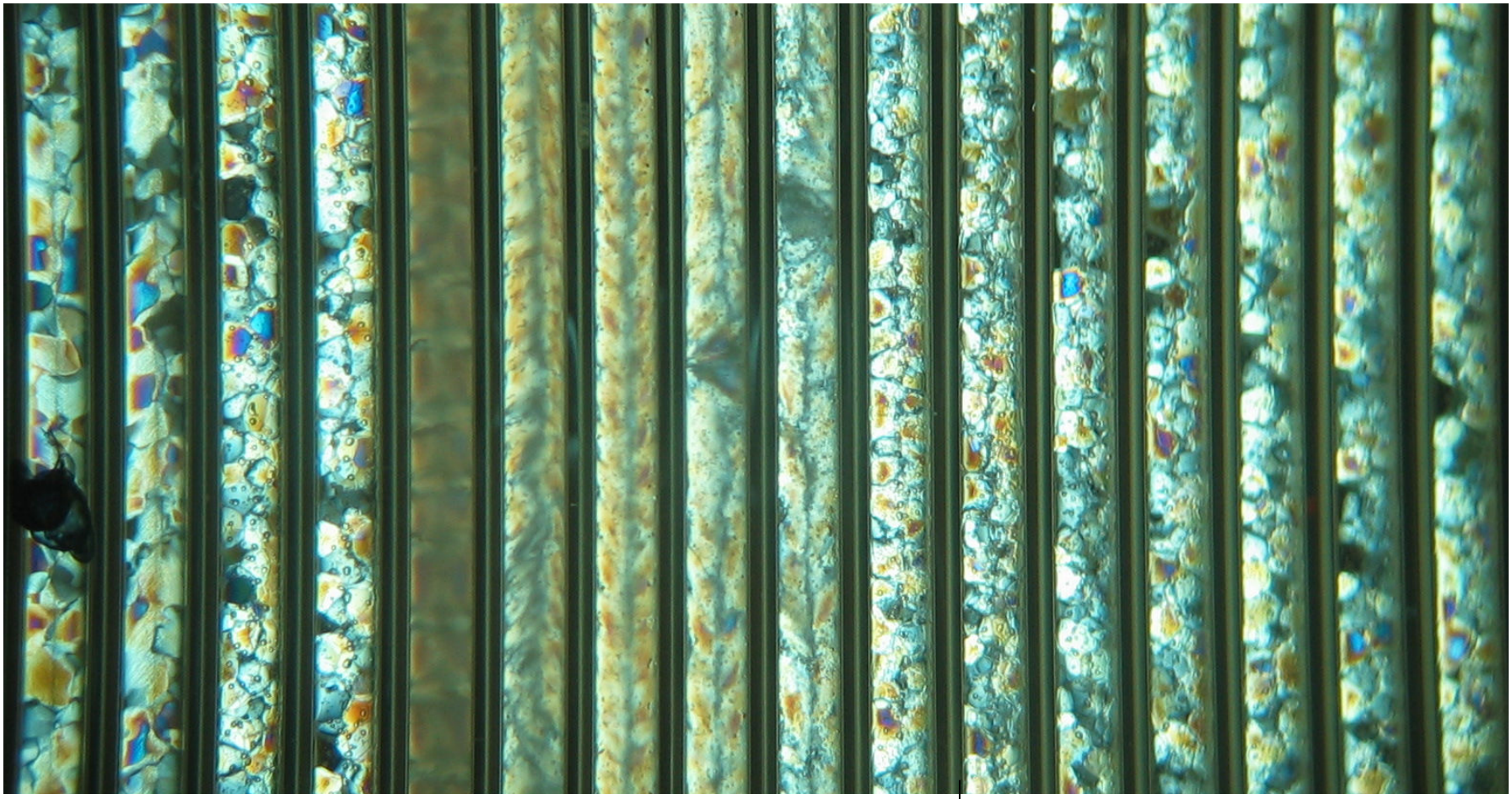


Why are these isolates so tolerant to freeze-thaw treatments? Could they have AFPs that inhibit recrystallization?



Ice recrystallization assay (18 h @ -6°C)

H_2O buffer AFP (decreasing conc.) BSA (decreasing conc.)

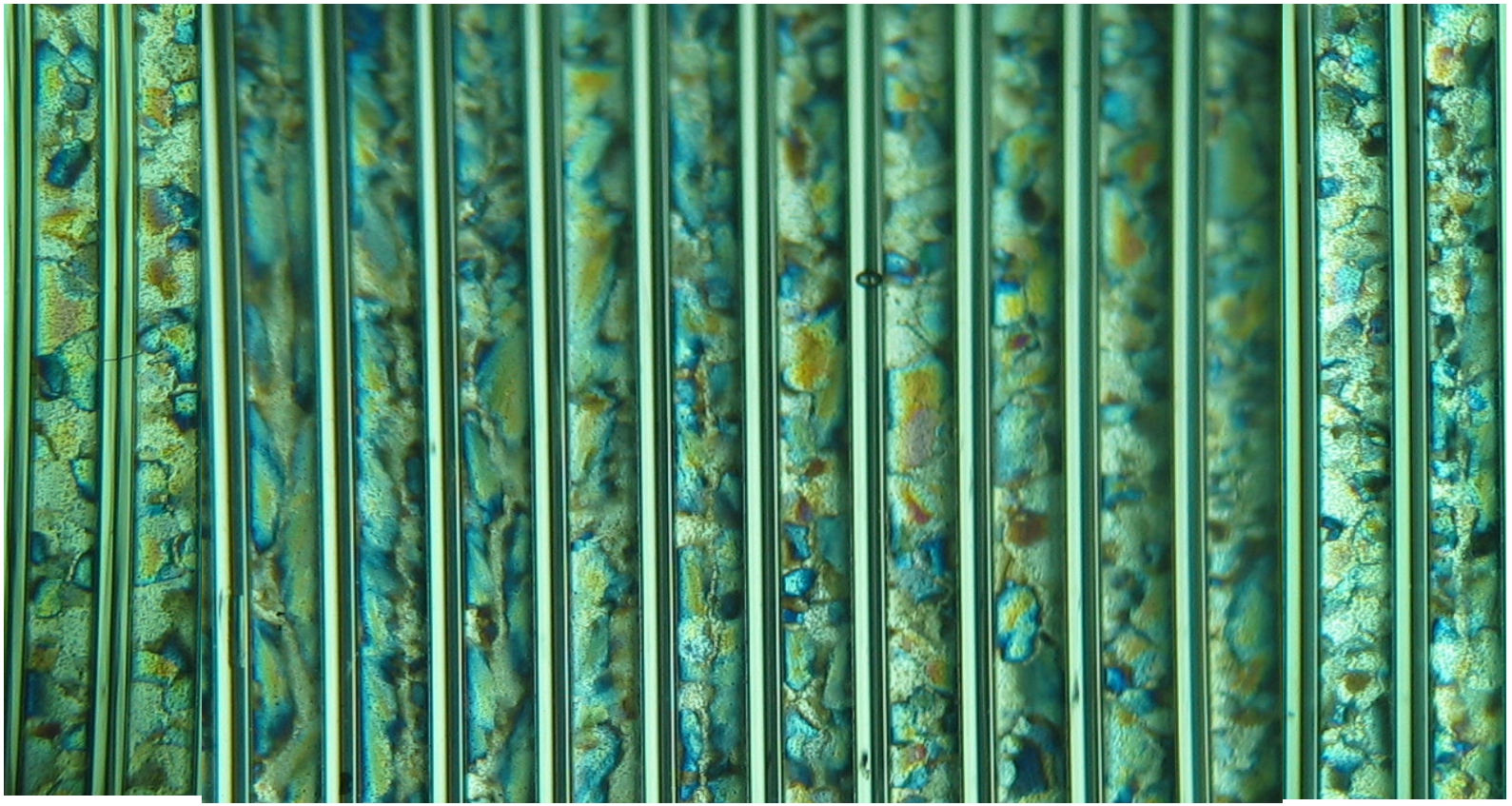


740 µm

mg/ml: 20 2 .2 .02 .002 .0002 20 2 .2 .02 .002 .0002

Inhibition of ice recrystallization assays with the selected isolates

E. coli	*Chyseobact.*	*Acineto.*	*Buttiaux.*	*Entero.*	*Carno.*



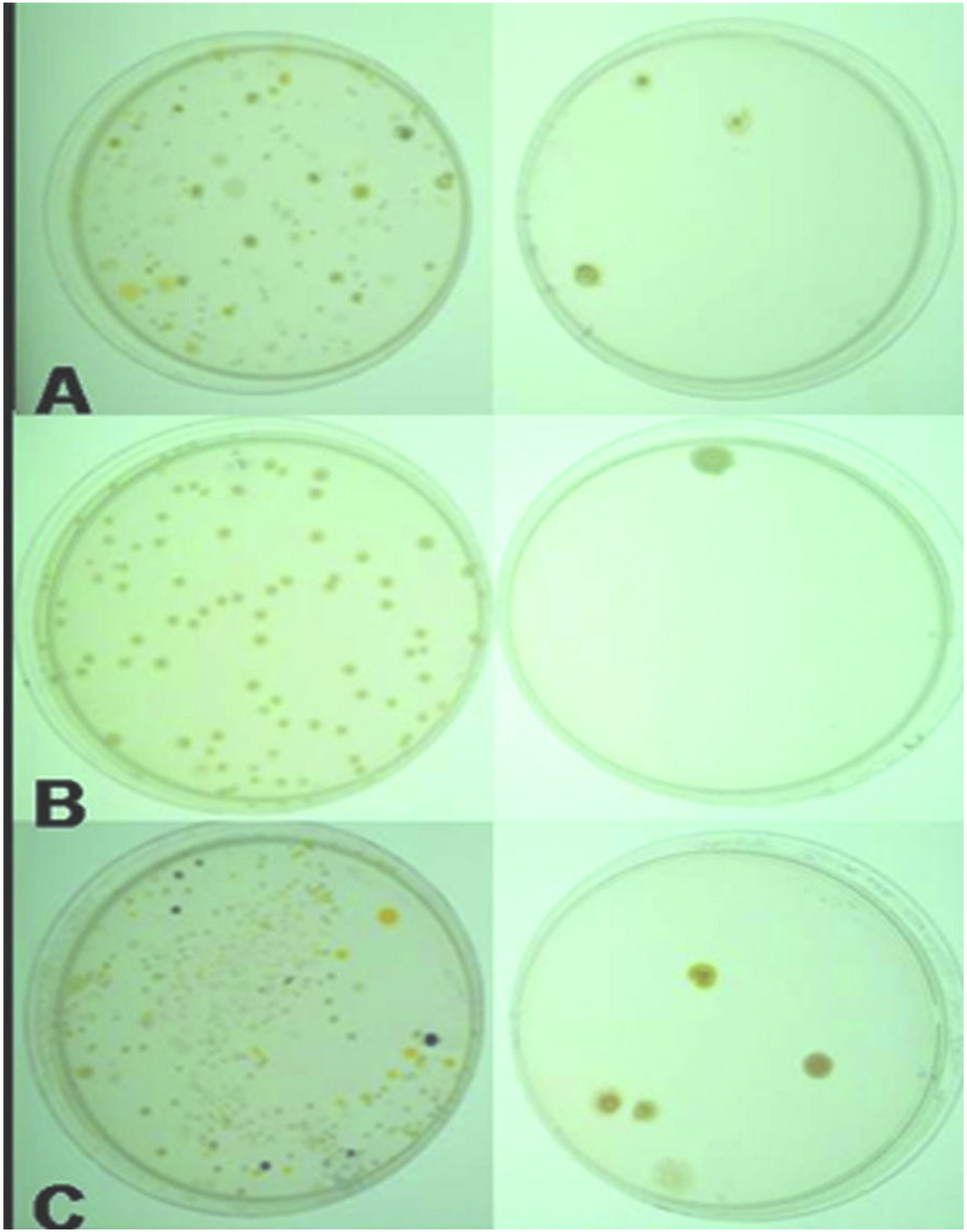
Would ice cream with microbial AFPs be a commercial success?

But expeditions to the Antarctic are expensive…can these microbes be found closer to home?

2. Selection using an "Popsicle"

Solutes are routinely excluded from growing ice unless they have an affinity for ice.

Can we select for psychrophiles from our back yards using a kind of growing Popsicle?

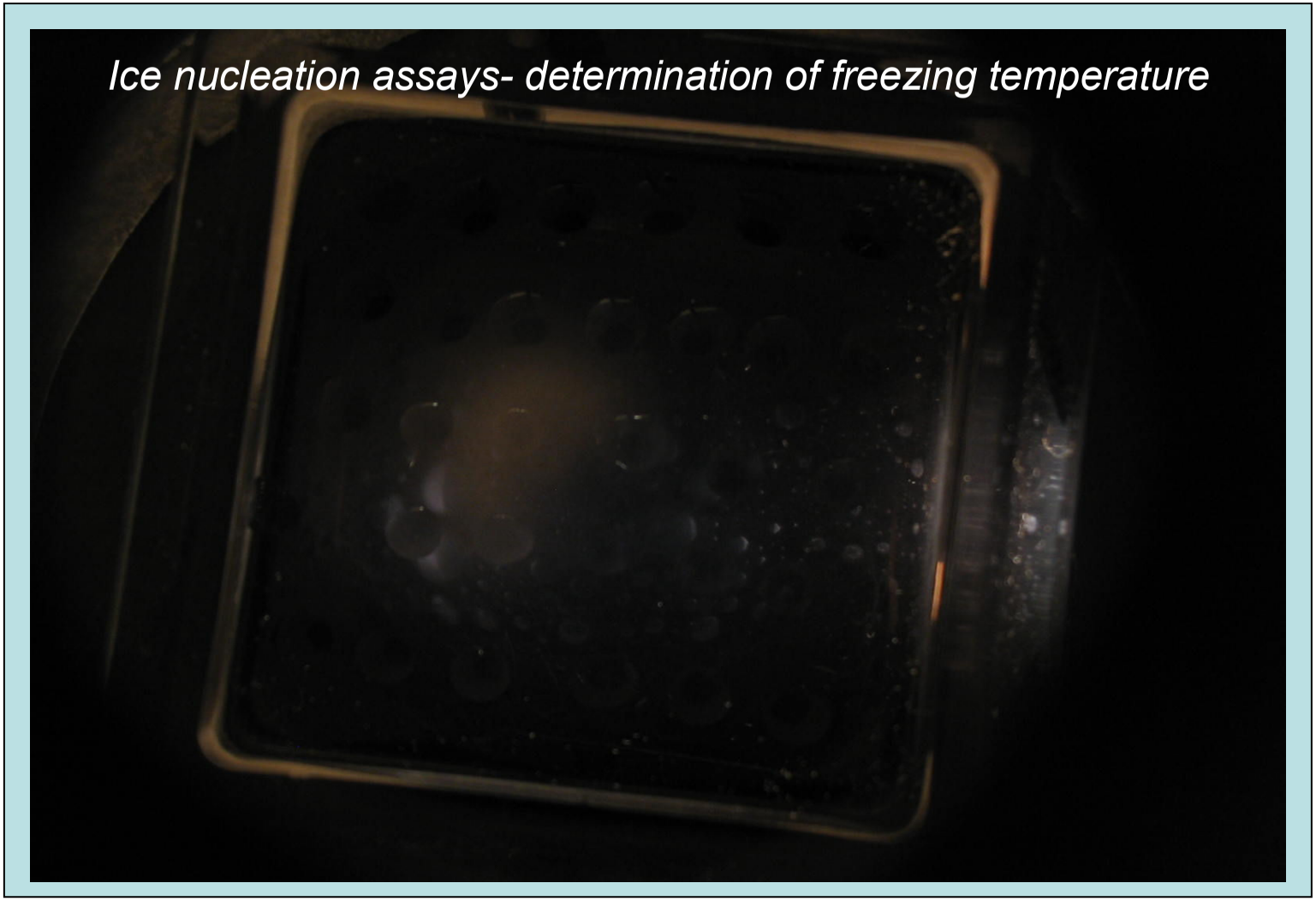


What did Sandra Wilson & Debbie Kelley find?

- 8 genera that have been previously recovered from glacial ice cores
- 3 genera that have been recovered from Antarctic lakes and several others from Greenland ice cores
- 2 genera were also recovered by cryocycler selection

Some of these microbes have ice-recrystallization inhibition activity and others have ice nucleation activity!

e.g. P. borealis DL7



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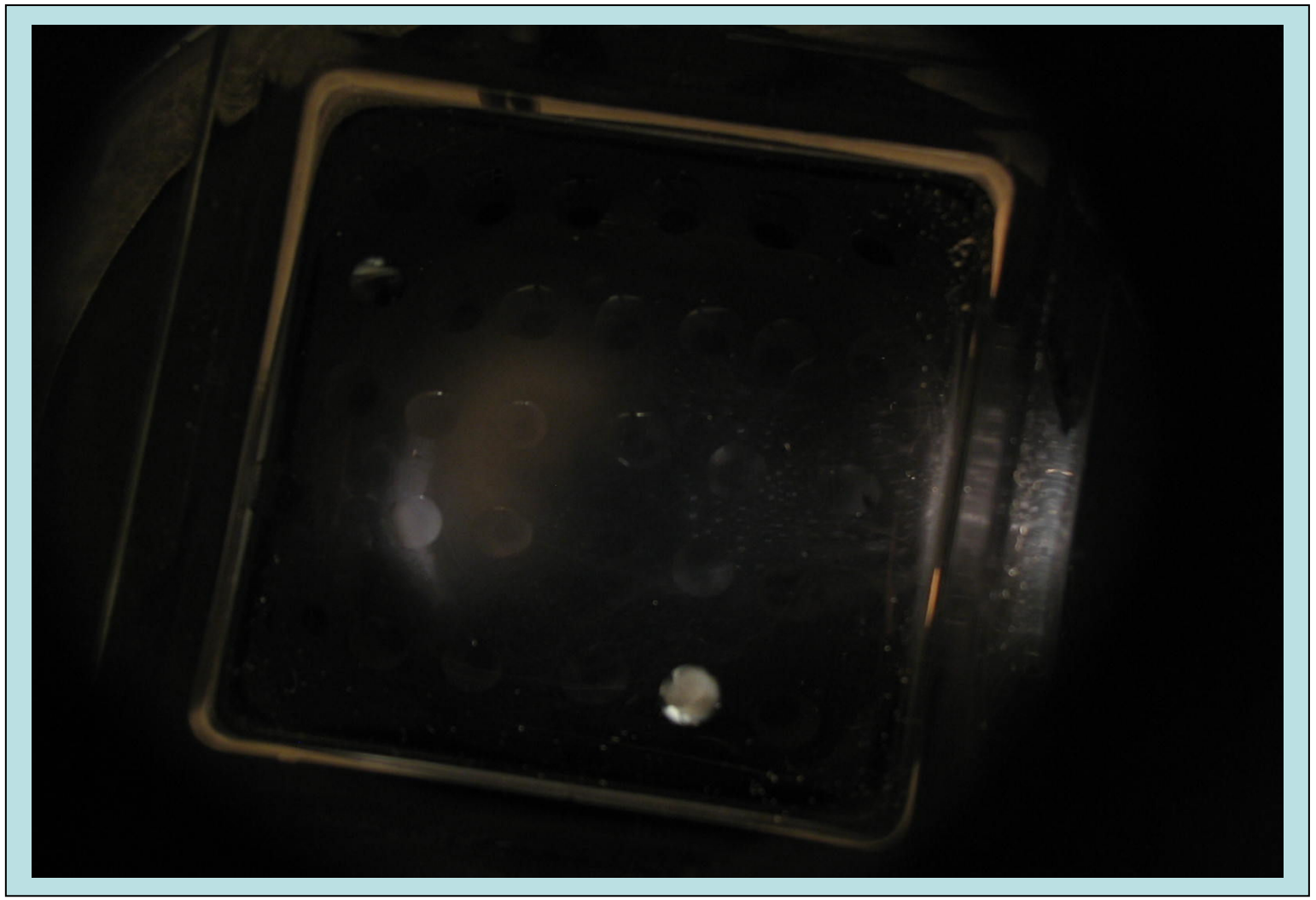

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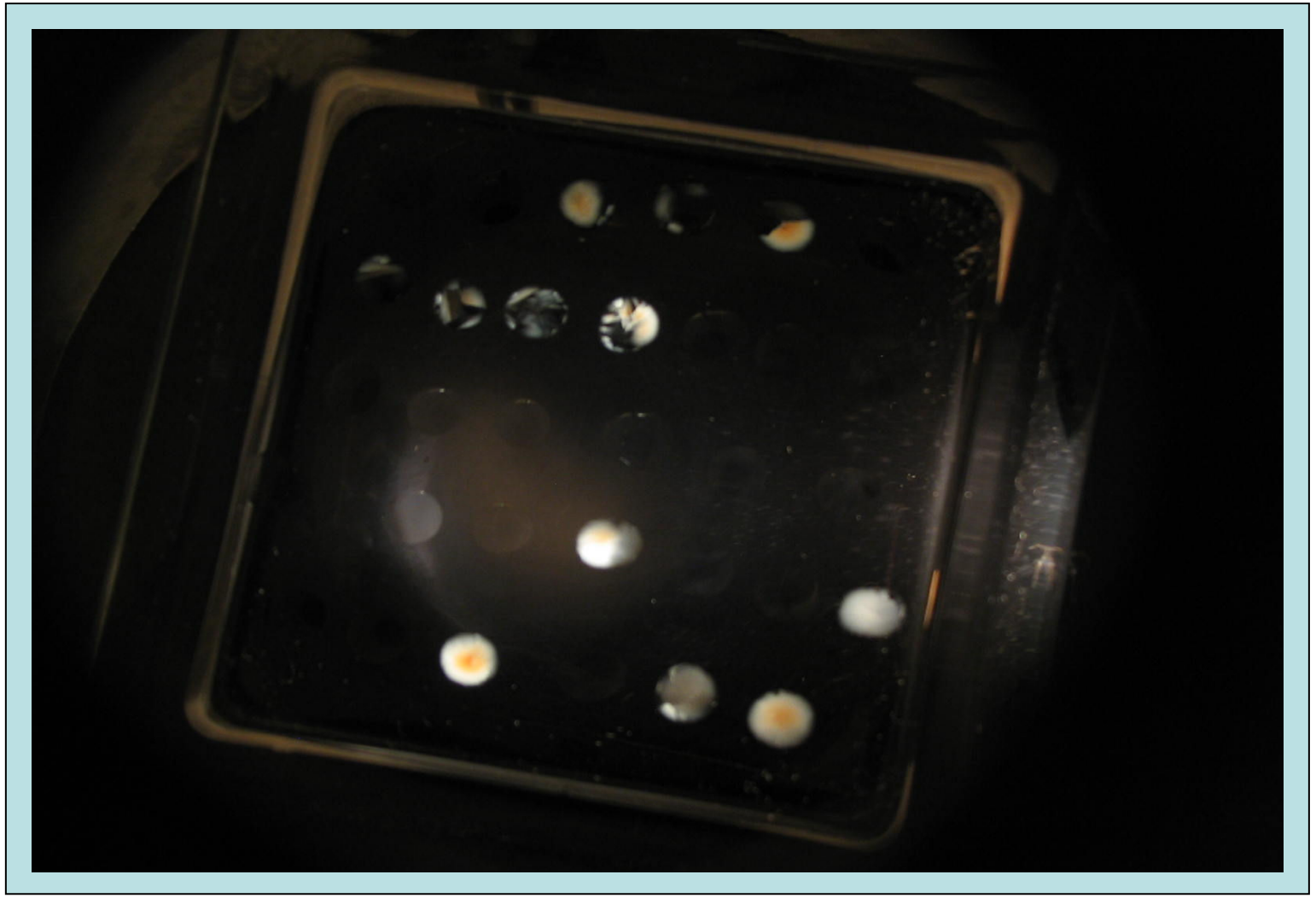

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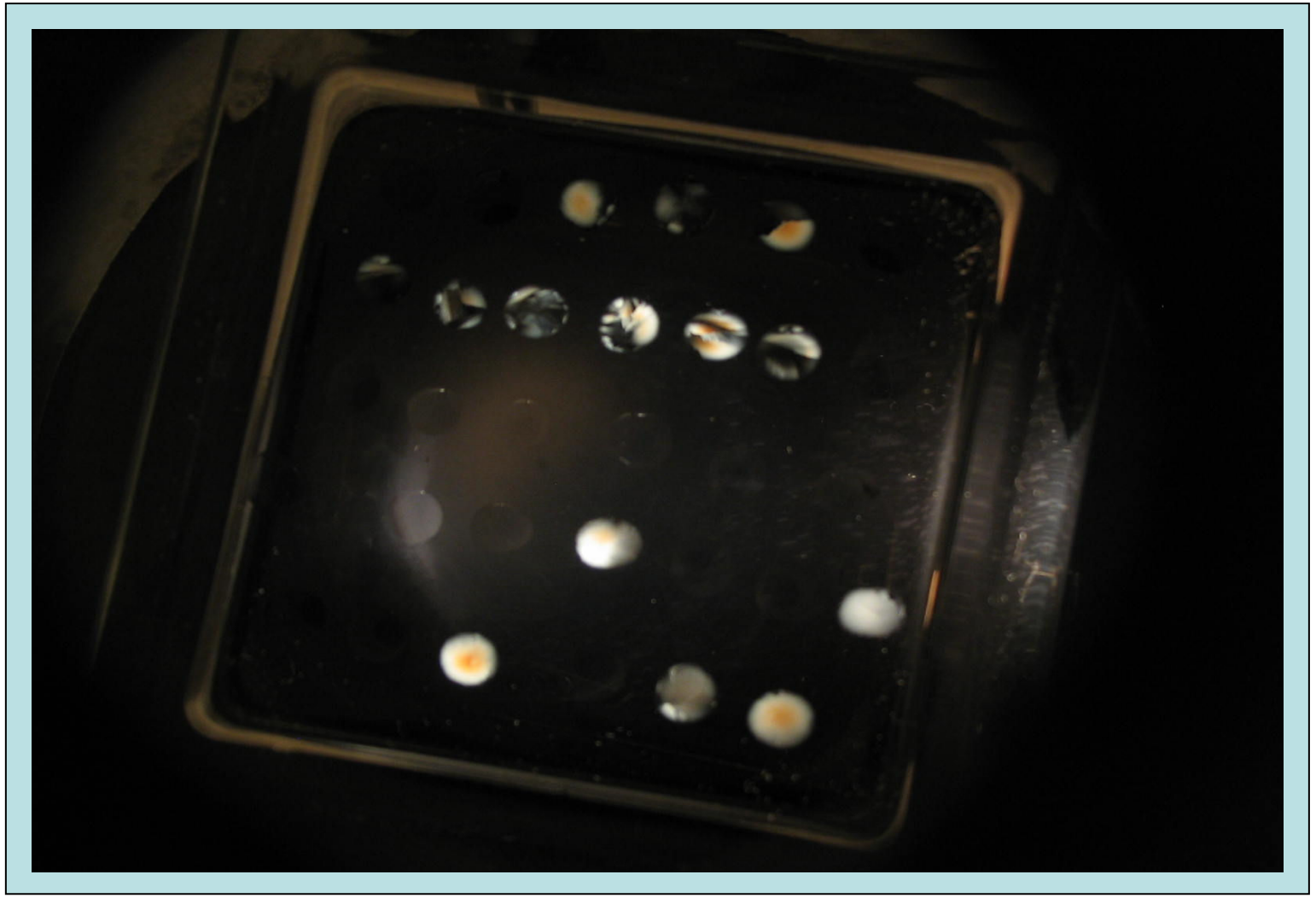

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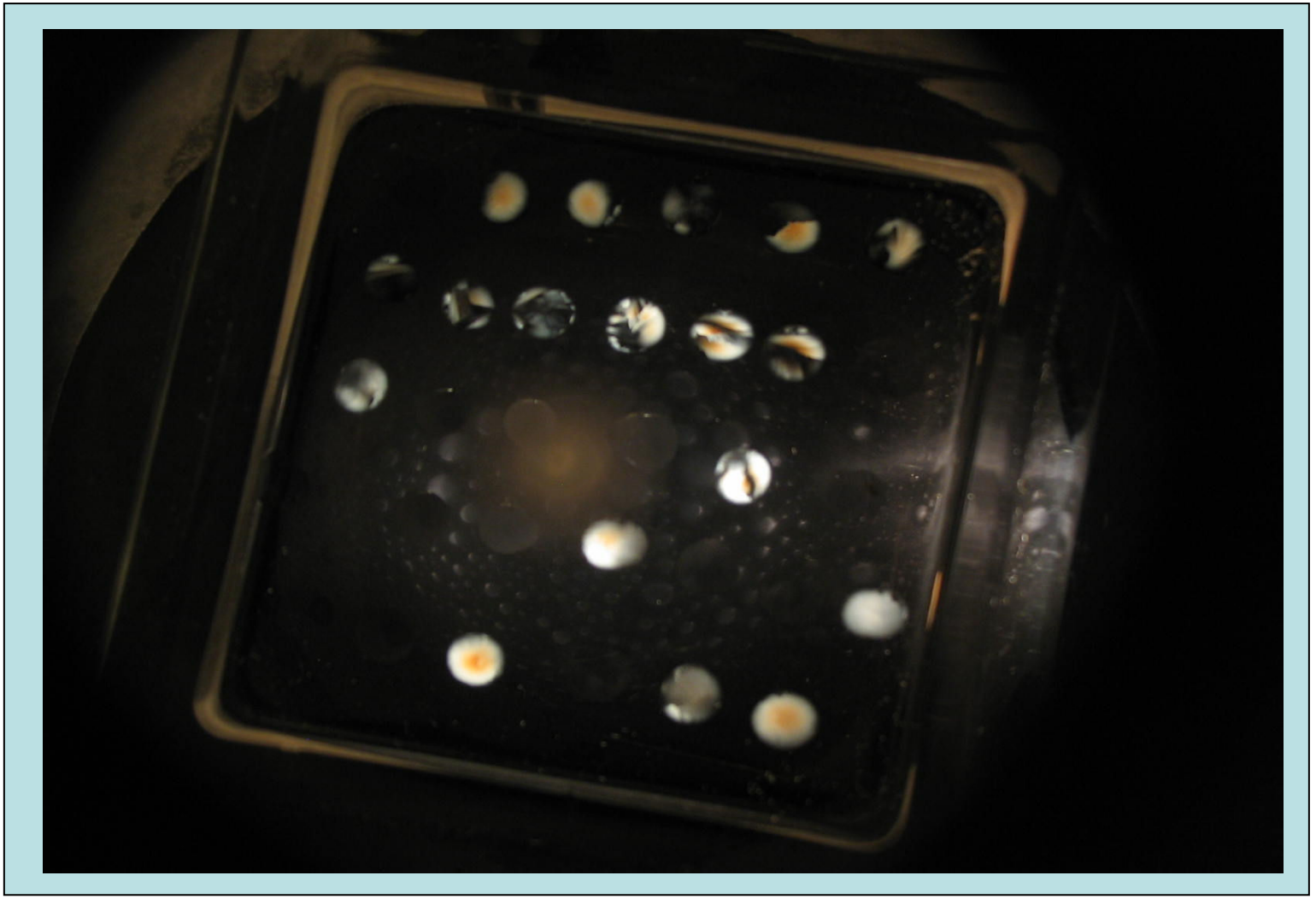

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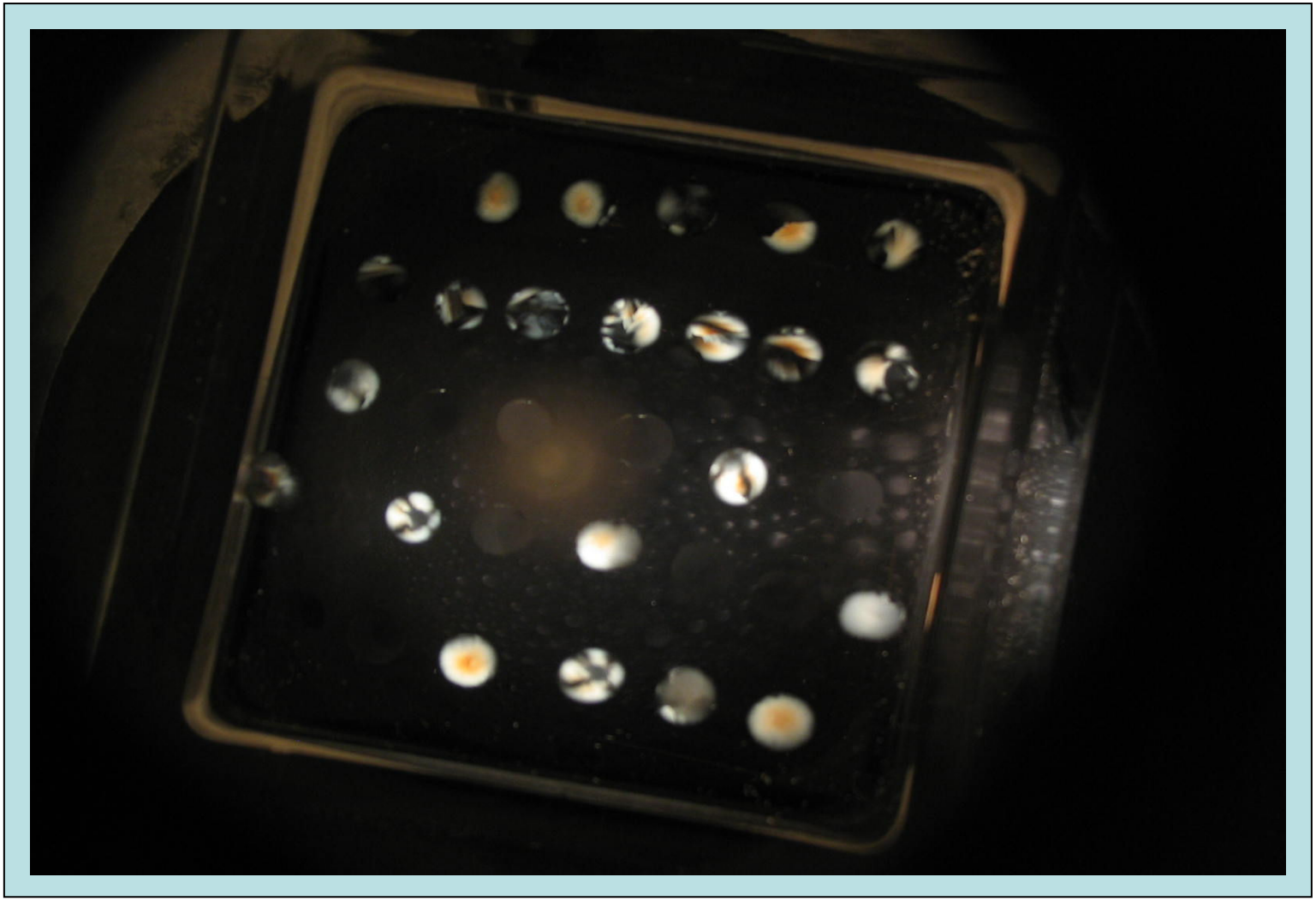

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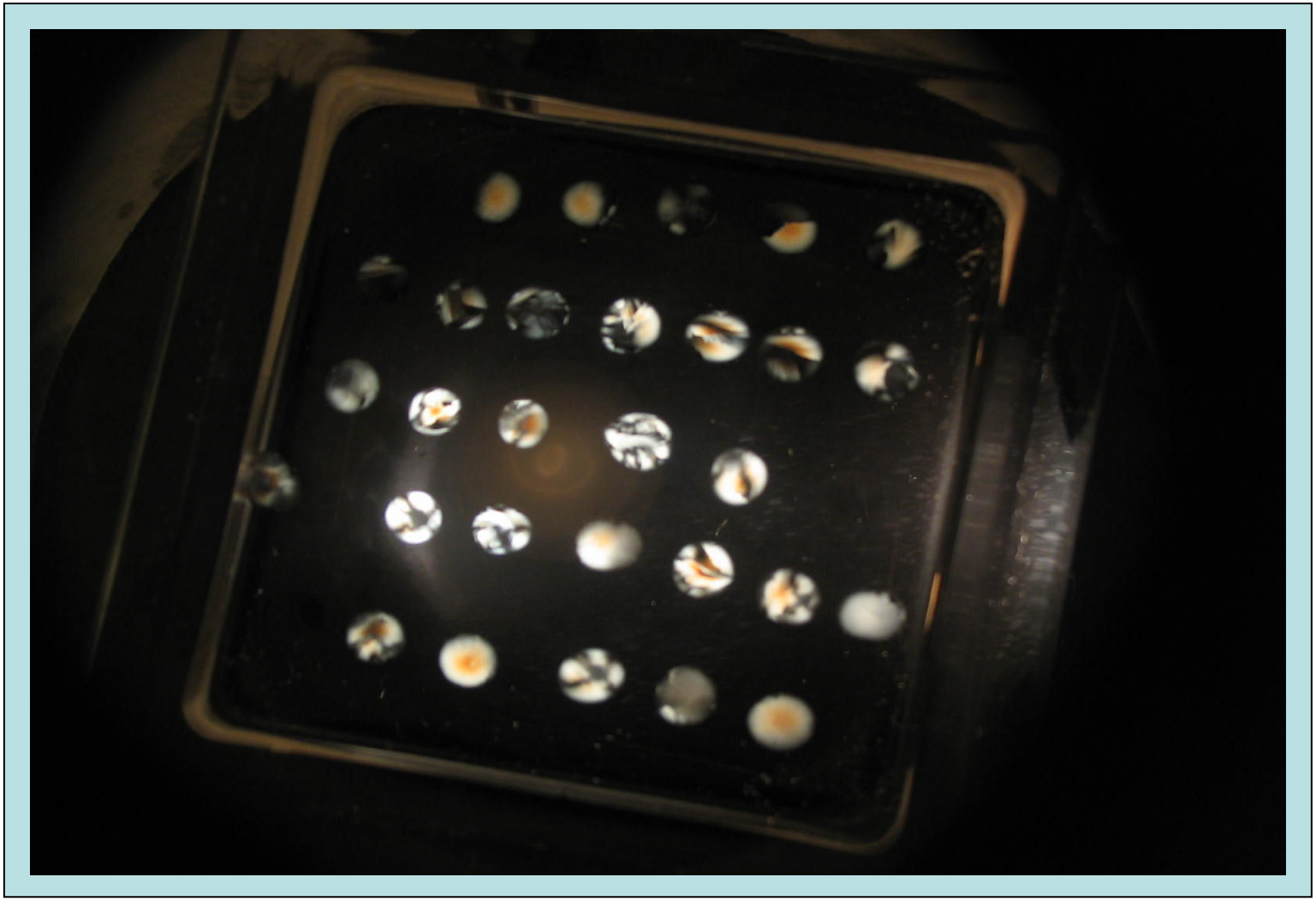
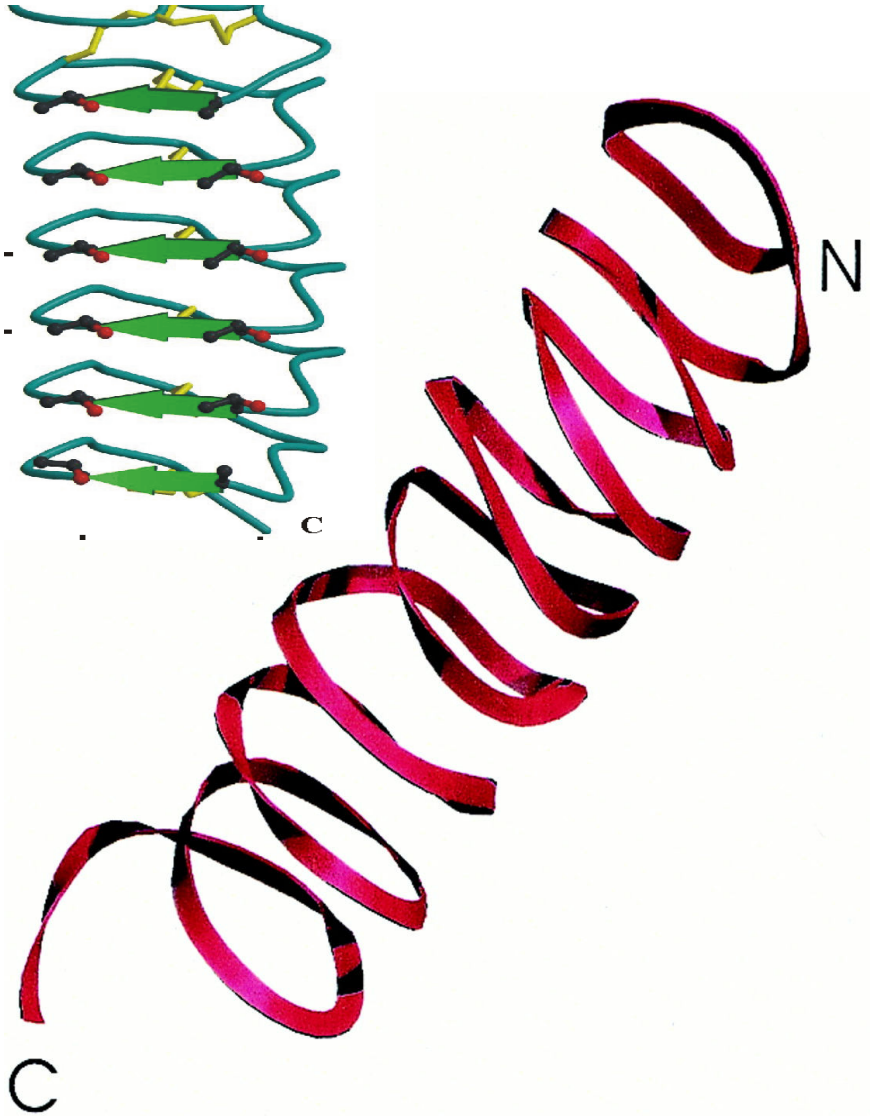
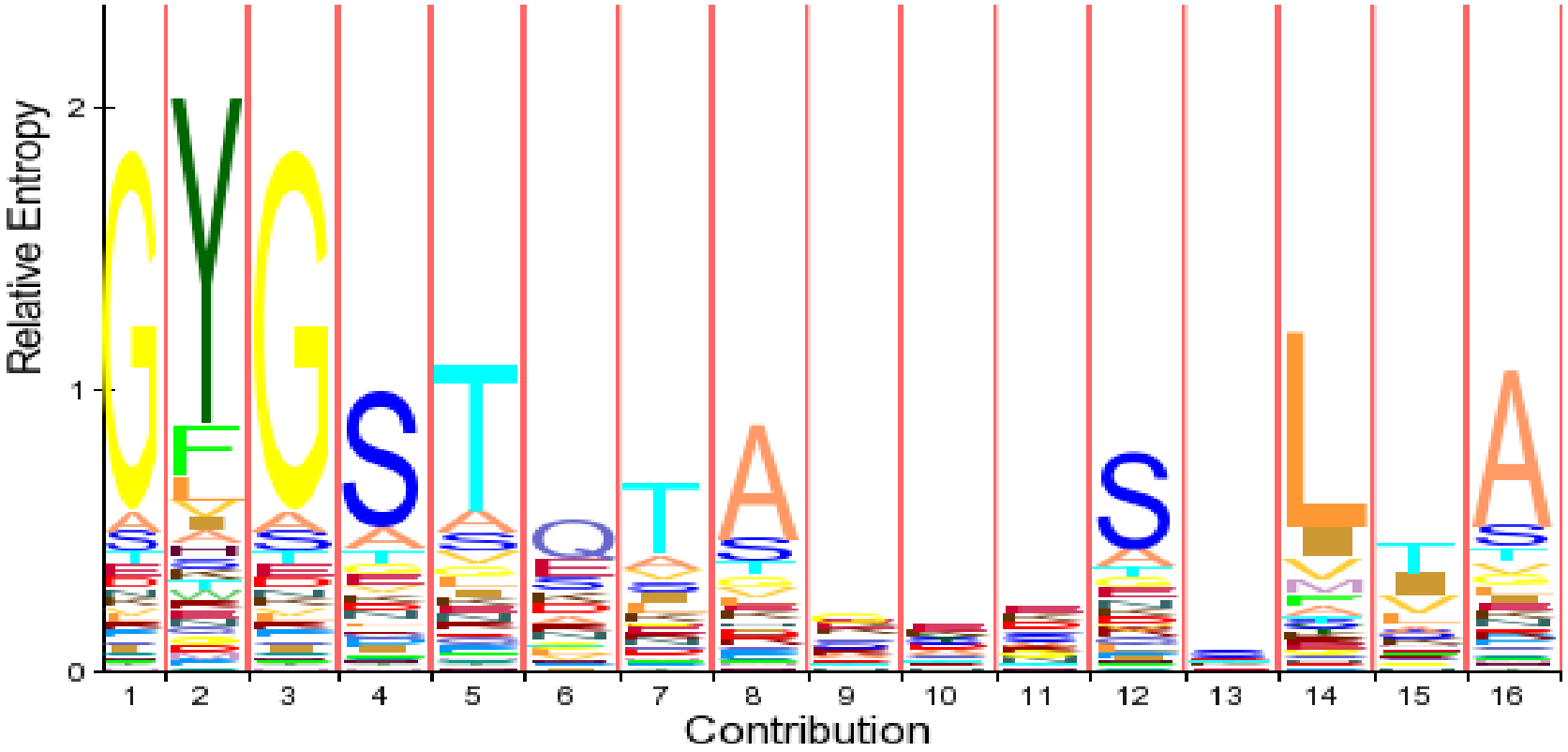


Antifreeze proteins *vs* ice nucleators

- AFPs lower the freezing point, but INPs don't allow solutions to supercool
- AFPs maybe smaller than INPs
- Some organisms may have AFPs and INPs <what?>

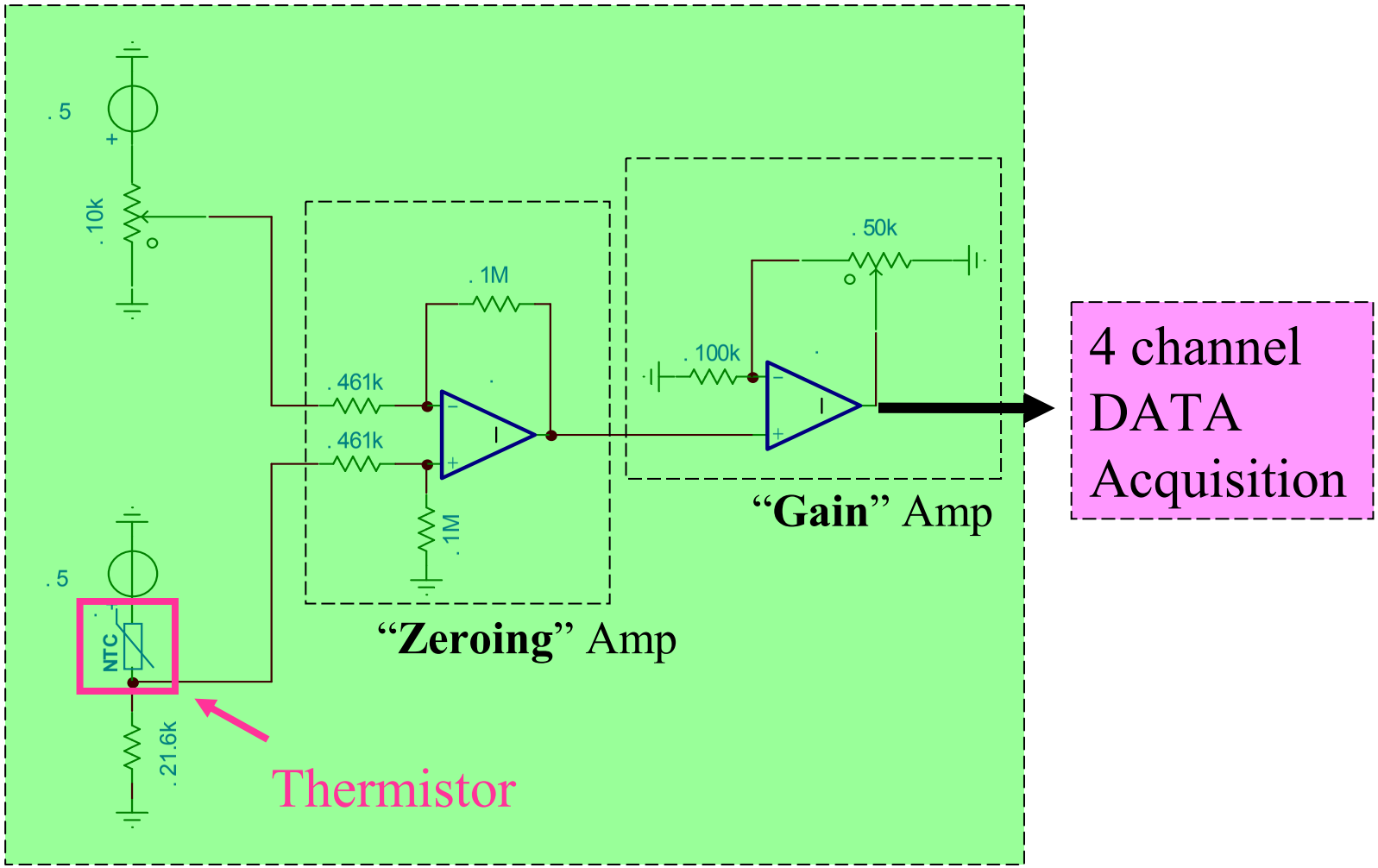


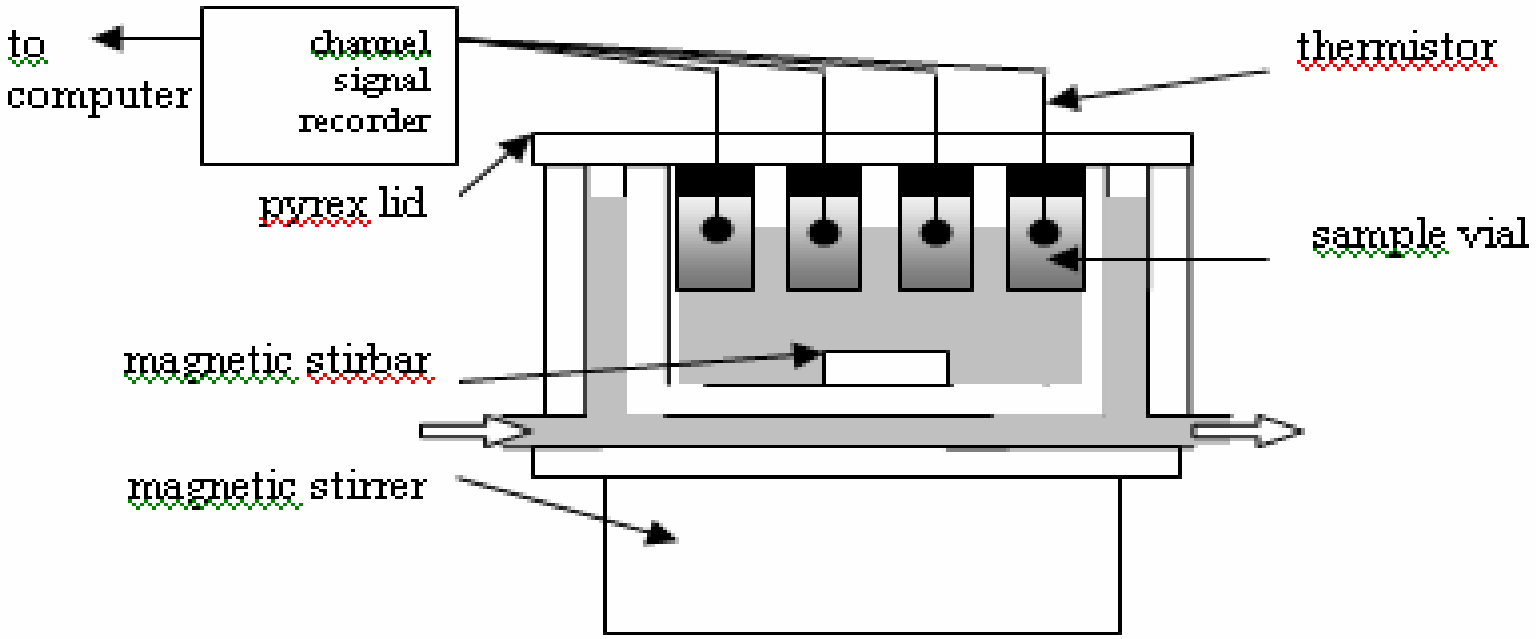
Suzy Wu in our lab has now isolated an ice nucleation protein gene from one of these microbes (the sequence from *P. borealis DL7* shows that it is repetitive)



The translated DNA sequence shows that it is composed of a 16 aa motif that is repeated along the protein. The frequency of amino acid use in the repeat region is shown by the size of the letters at each position.

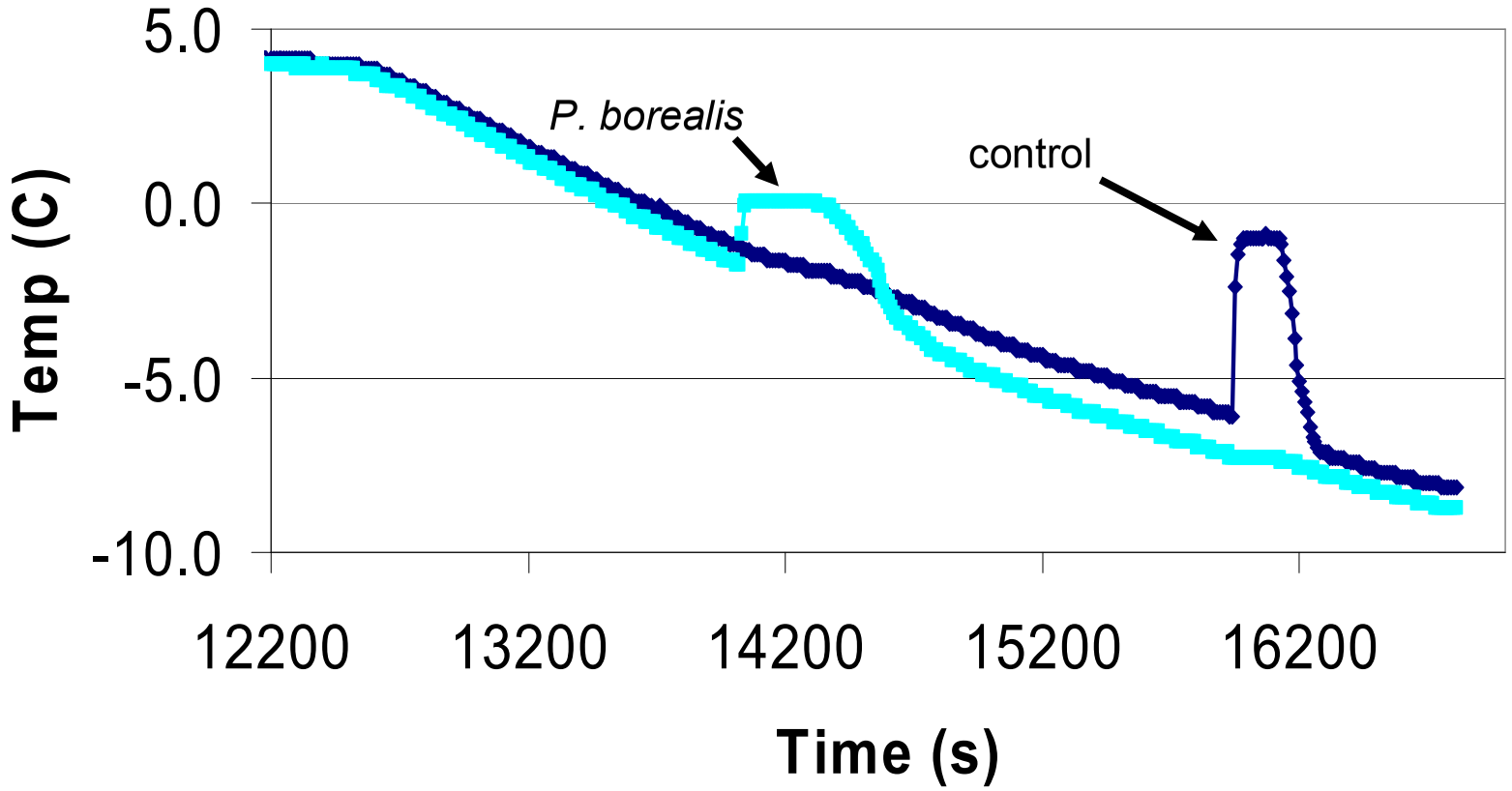
Temperature DATA Acquisition- let a computer do the watching….



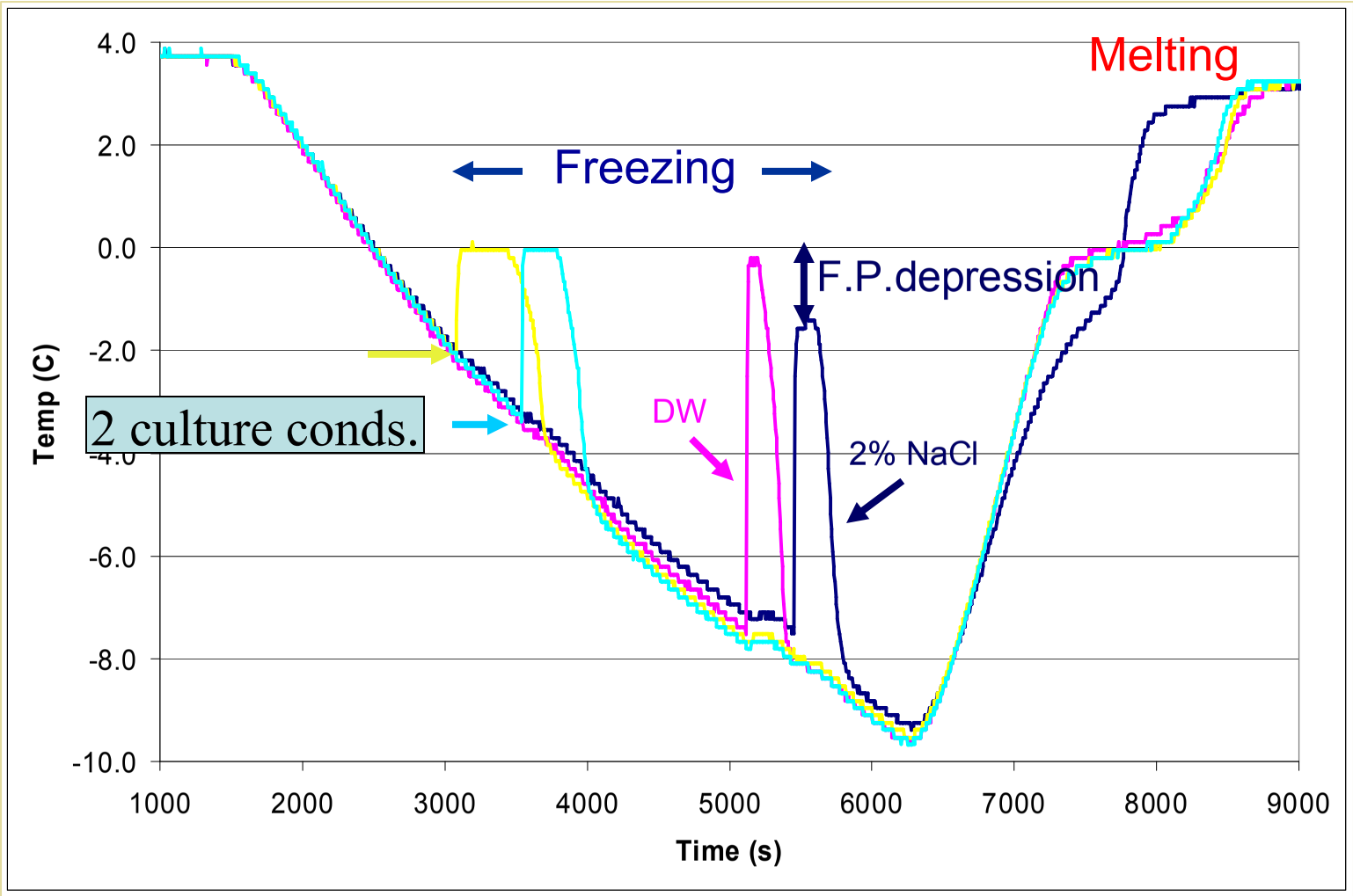


Recall that when solutions freeze:
(i) they release heat (heat of crystallization)
(ii) then decrease in temperature taking into account Newton's law of cooling

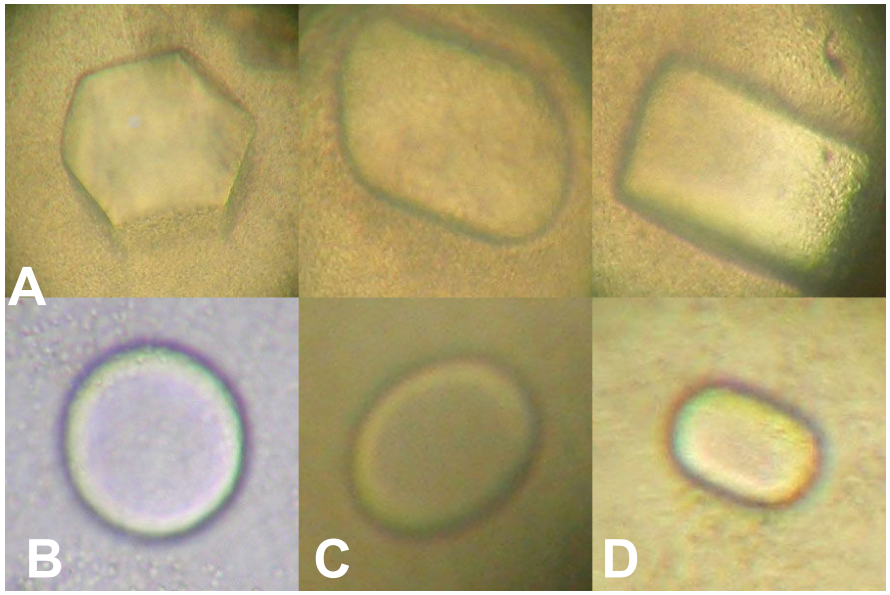
Pseudomonas borealis was isolated on the Popsicle and it has a protein that acts as an ice nucleator (Suzy's gene).



Ice nucleation activity of *P. borealis* grown at 22ºC and 4ºC



Some too, show ice shaping



A. *P. borealis* DL7

B. *A. radioresistens* DL5

(*P. syringae*, *Paenibacillus amyloliticus* C8, *E. coli*, INPs, TSB)

C. *Chryseobacterium* sp. GL8

D. *Flavobacterium* sp. GL7

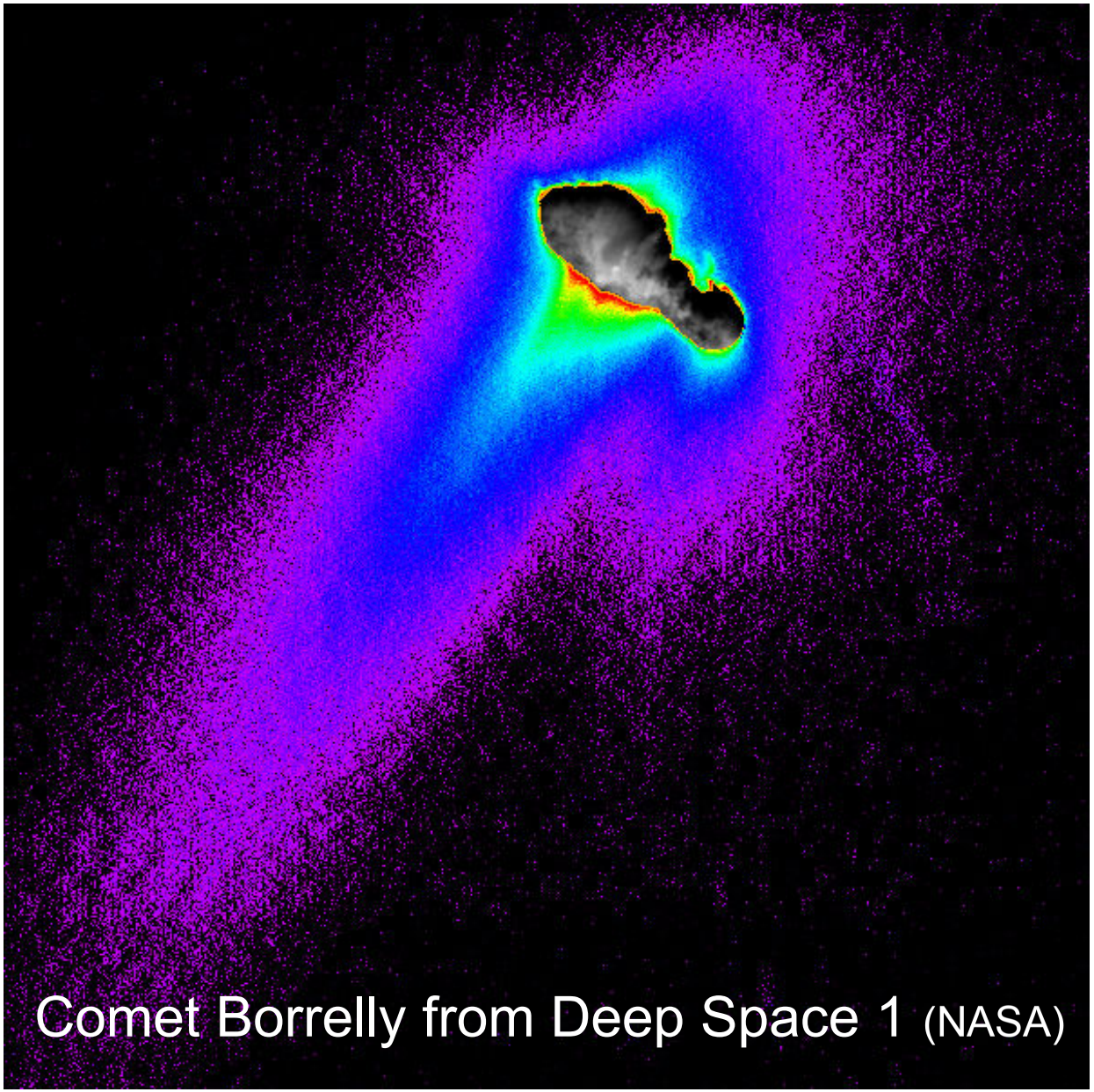
(*Chryseobacterium* sp. C14, at 4°C for several days)

What about Mars?

Some (including a team from the Goddard Space Flight Center) have speculated that Mars may have methane hydrate, perhaps on the polar caps.

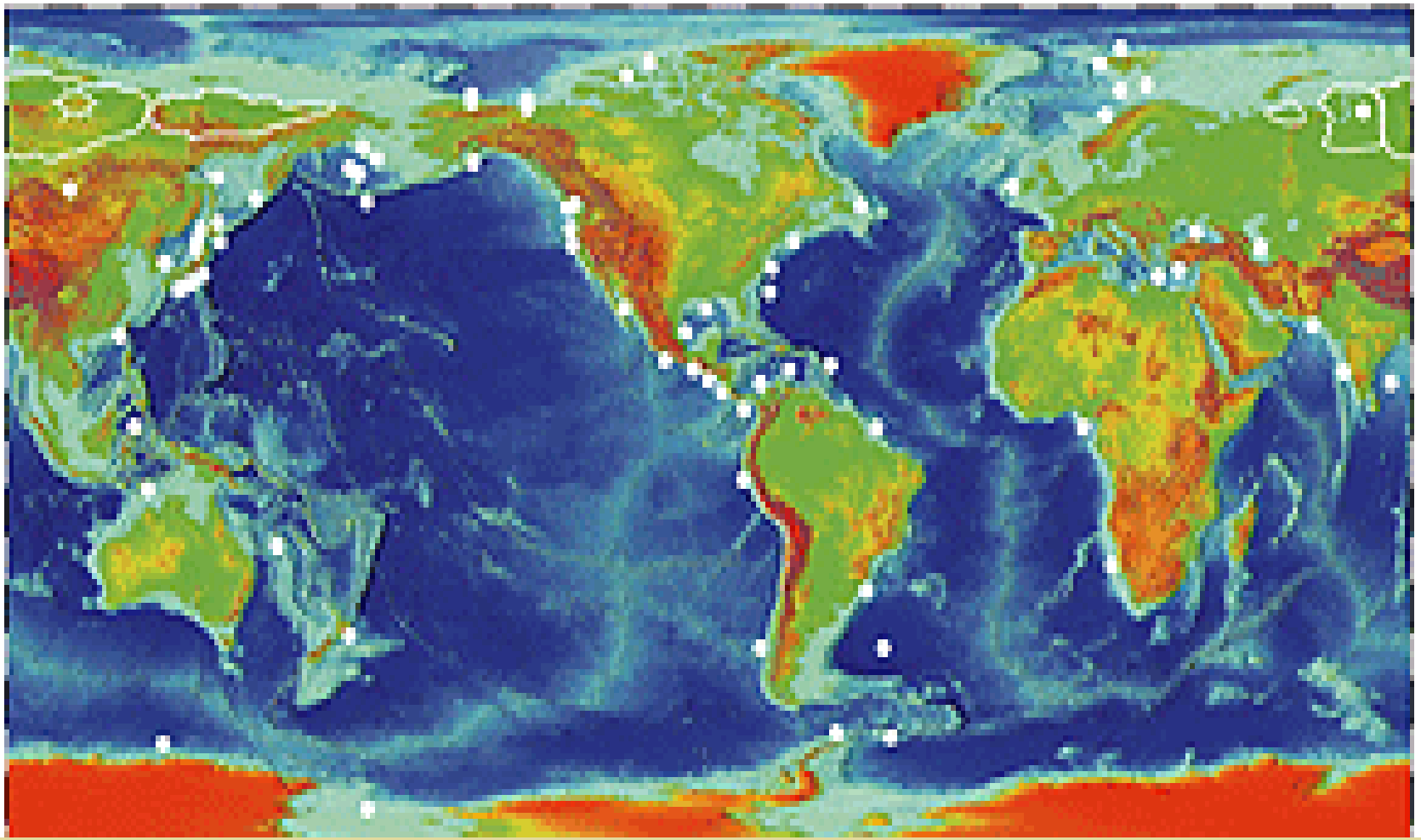
Could life be found associated with the hydrate?

methane, ethane, CO hydrates in comets?

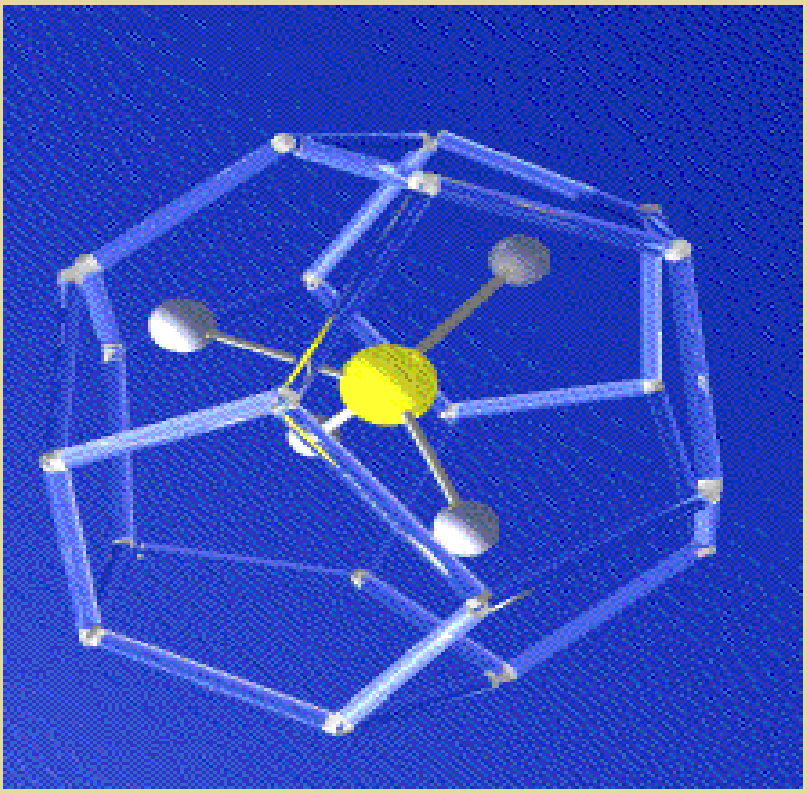


“Please, Virginia do we have to stay in the -20°C freezer room to research AFPs?”

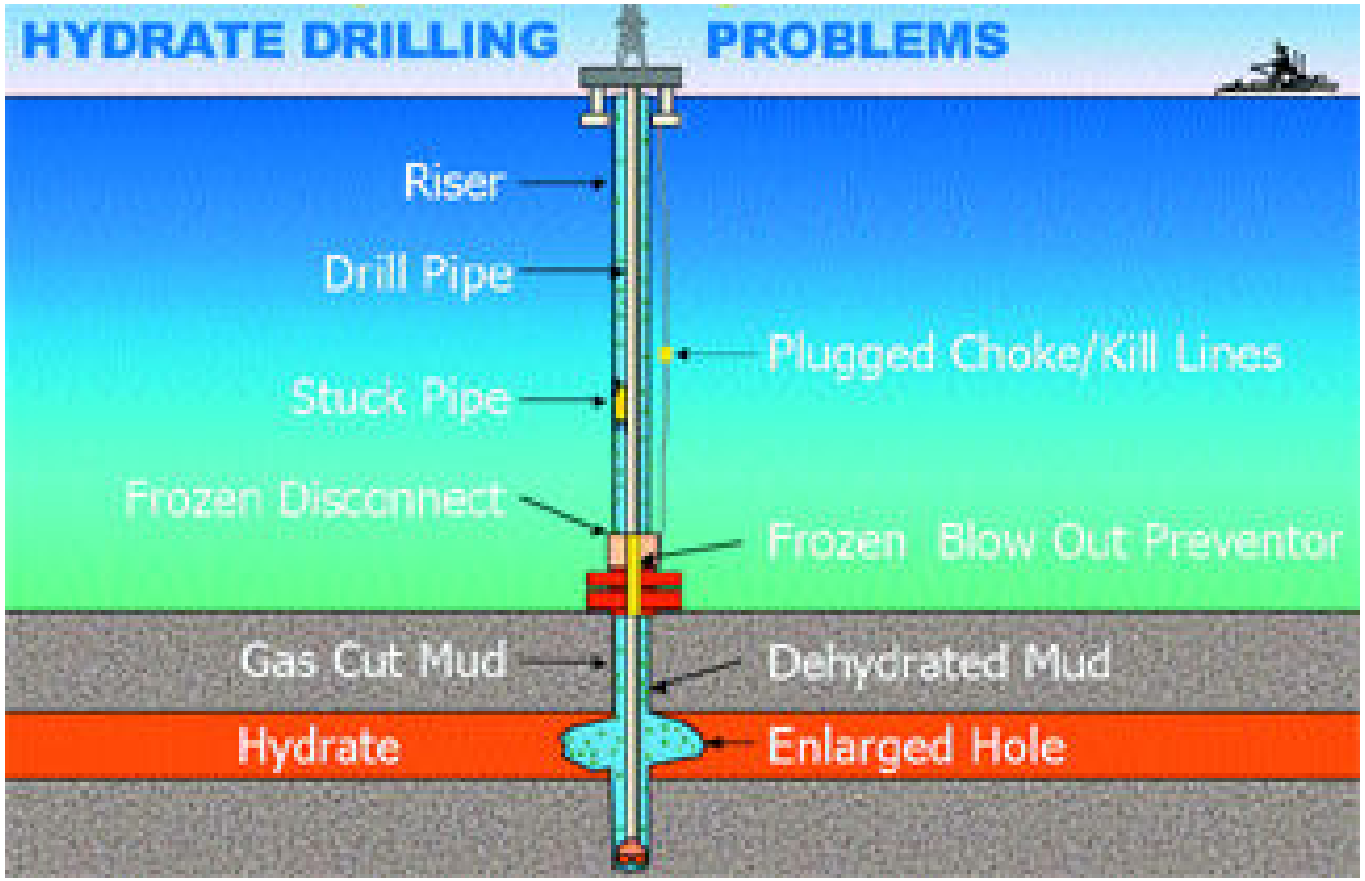
On earth, gas hydrates are found around the continental margins



Some hydrates have been formed by microorganisms, others by geological processes



Gas hydrates- the good and the bad…
future energy source and safety/environmental concern

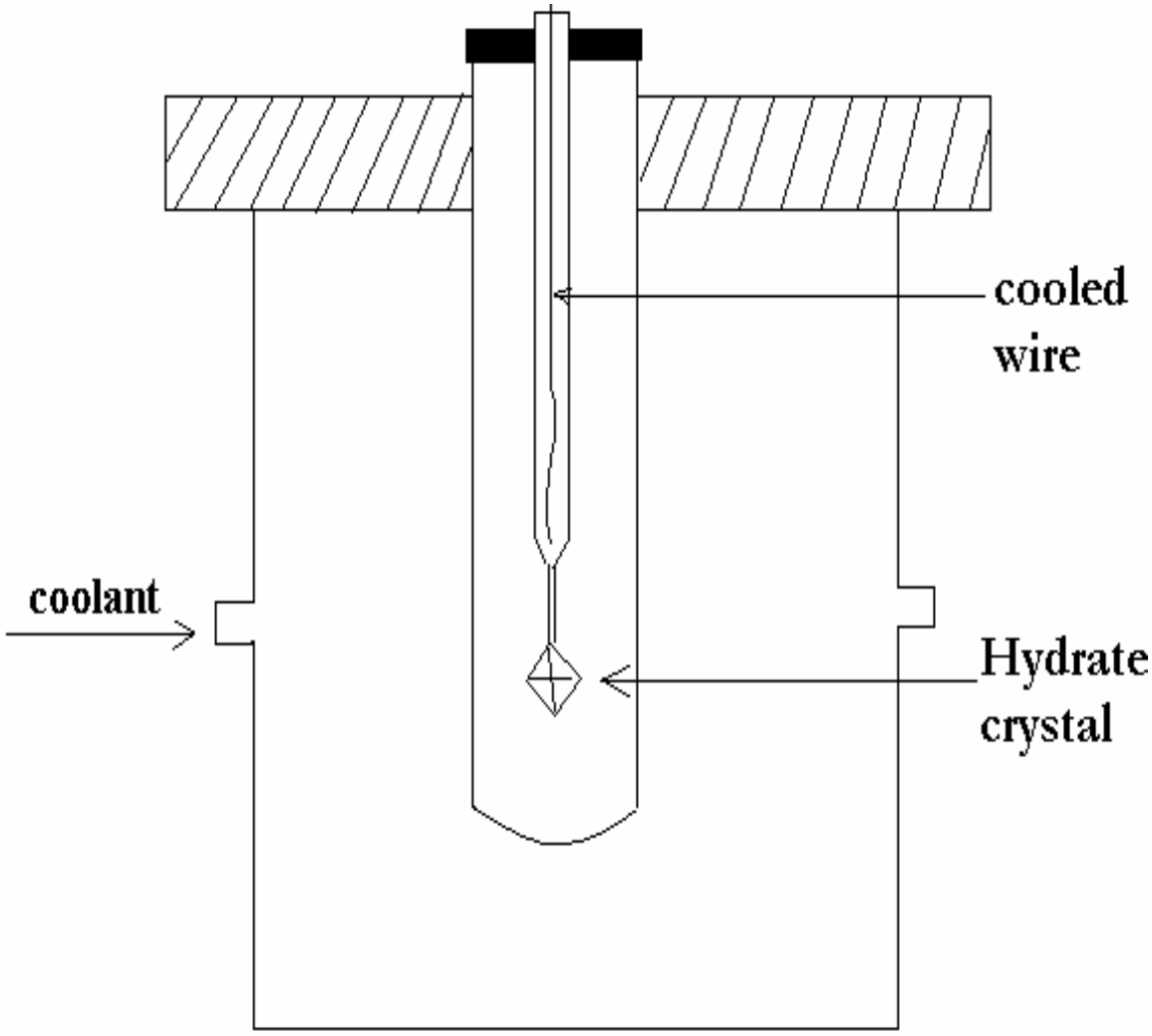


It is estimated that there are 10 000 gigatons of carbon in ocean gas hydrates (vs. 700 gigatons atmospheric carbon)
http://www.geotimes.org/nov04/technology.html

Hydrates can form spontaneously when drilling for natural gas, leading to blocked pipes and sometimes, disaster

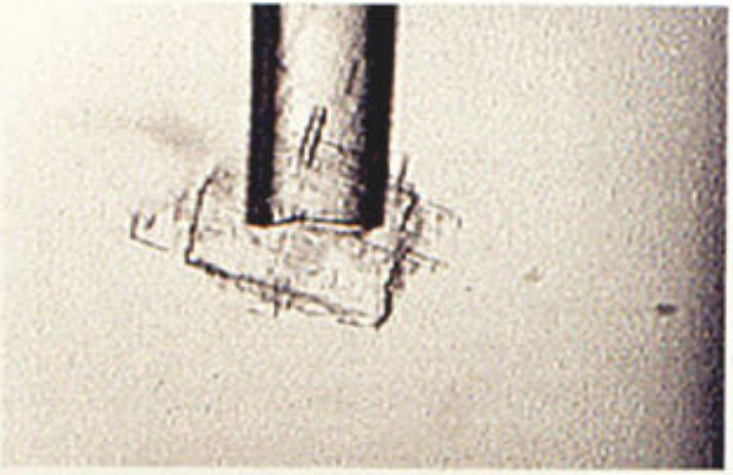
Piper Alpha blowout in the North Sea resulted in the deaths of 167 oil workers

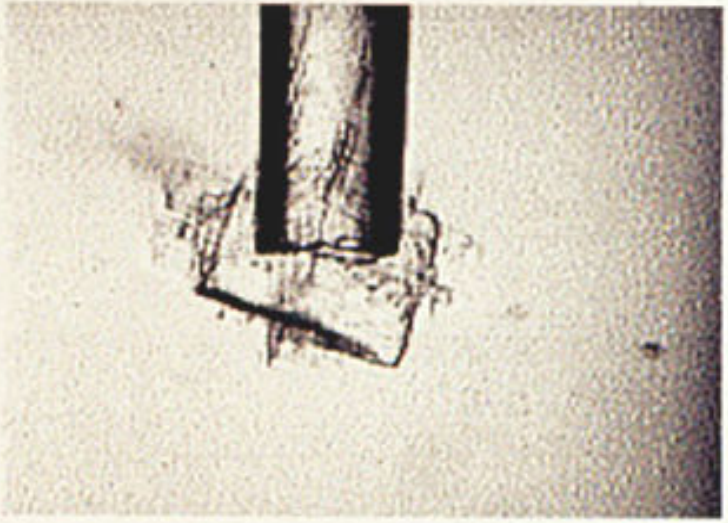
Huang Zeng's work: growing a model gas hydrate in our laboratory



Inhibition of gas hydrates by AFPs

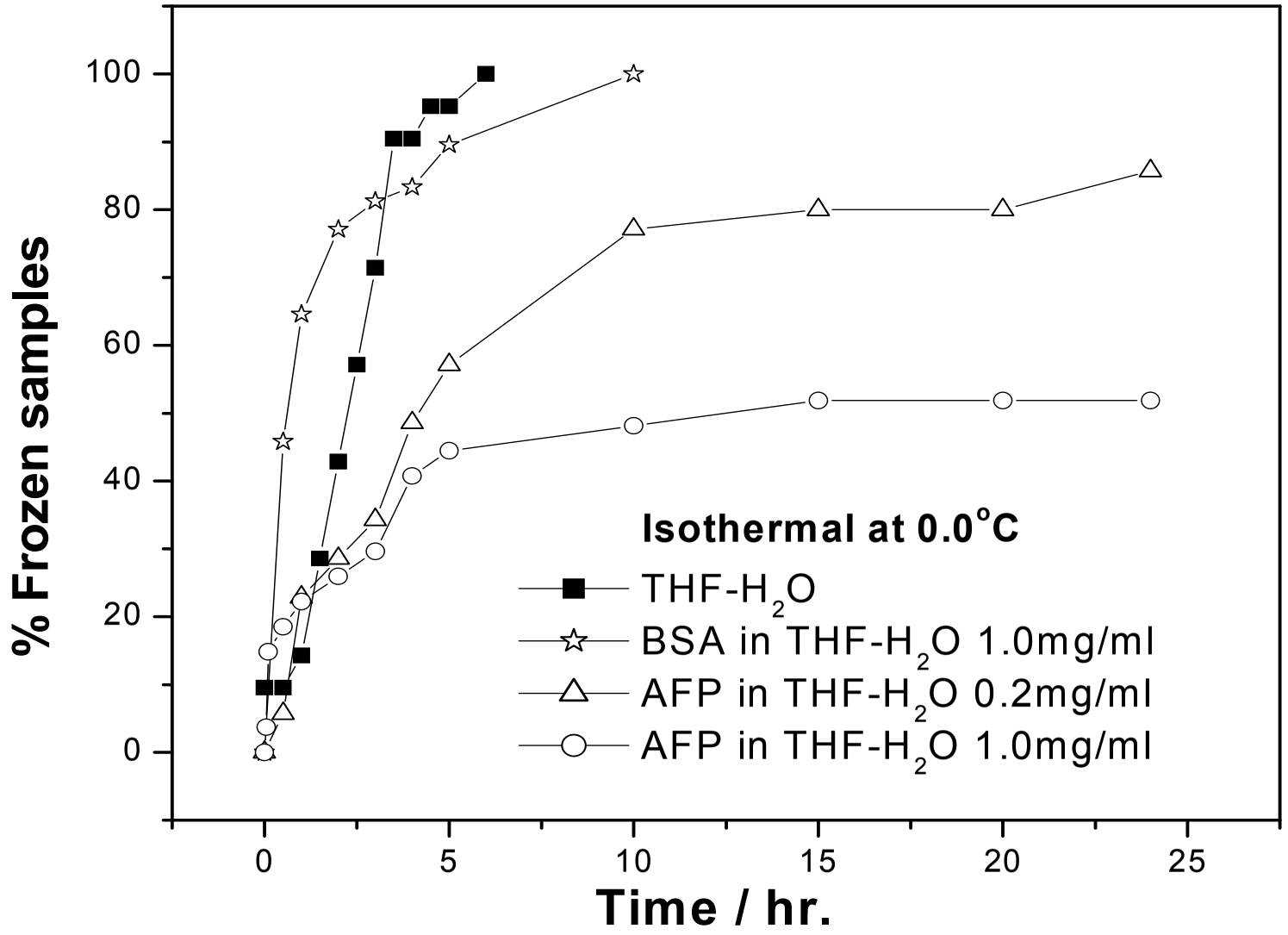
Octagonal THF crystals (no AFP)

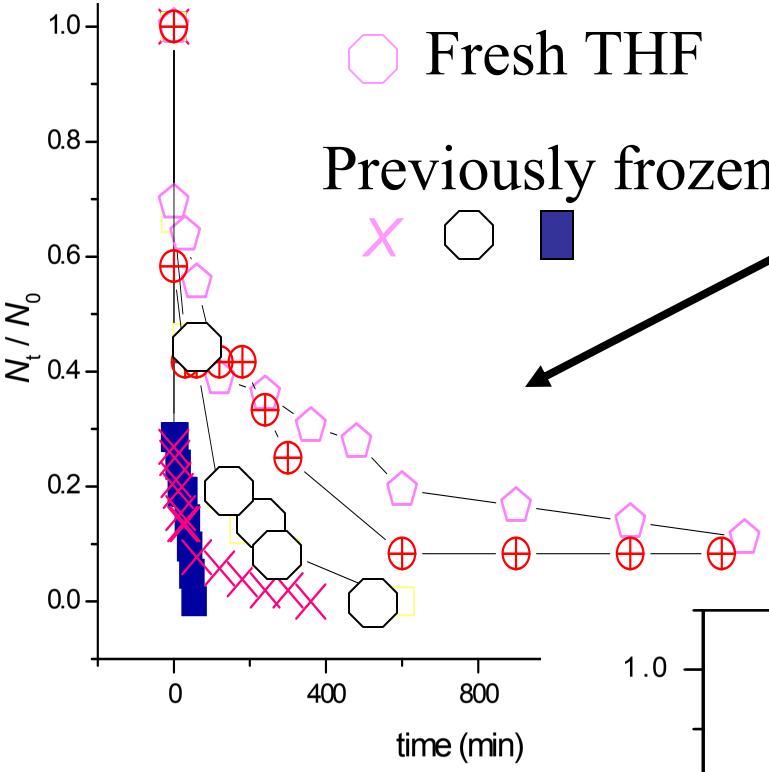




Small, plate-like crystals after transfer to AFP

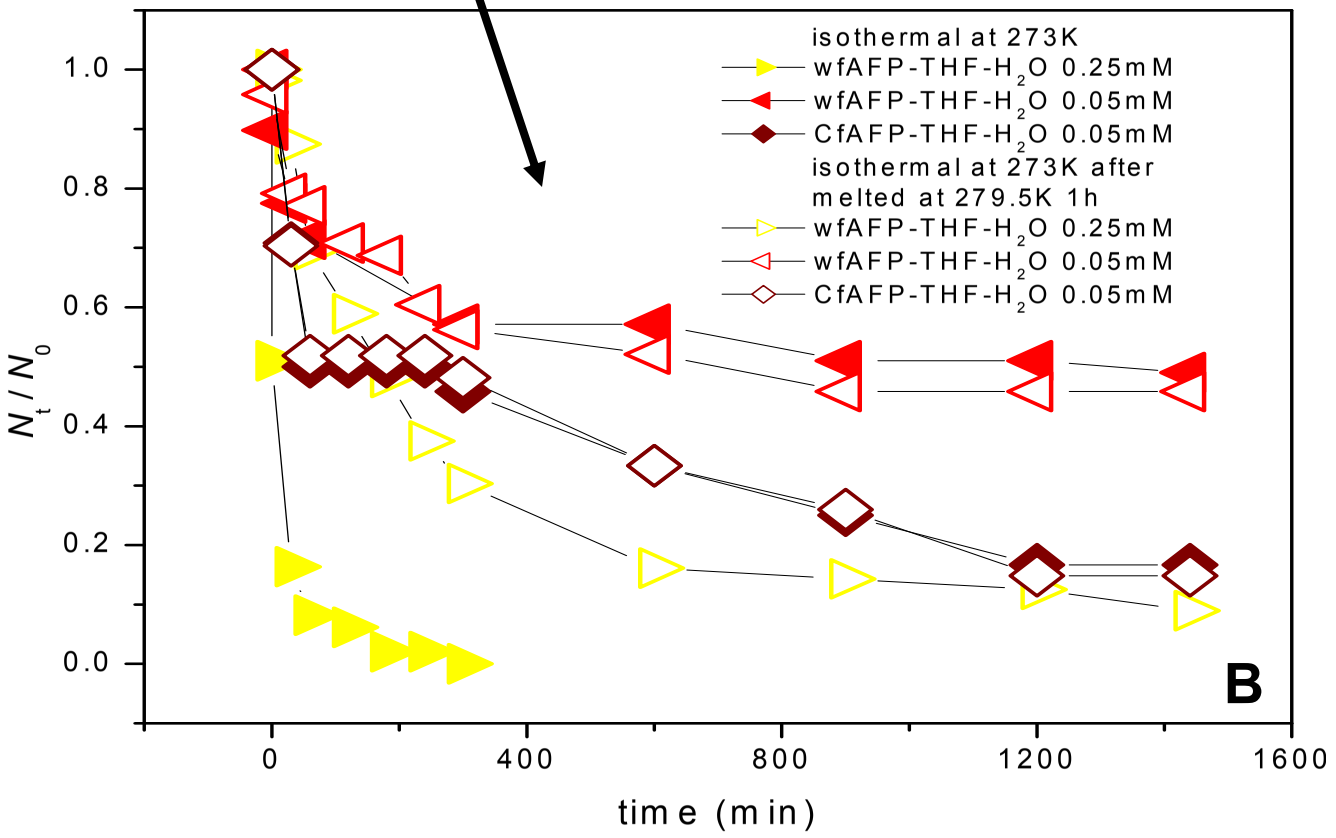
It takes much longer for samples with AFP to crystallize- therefore AFPs are hydrate inhibitors!





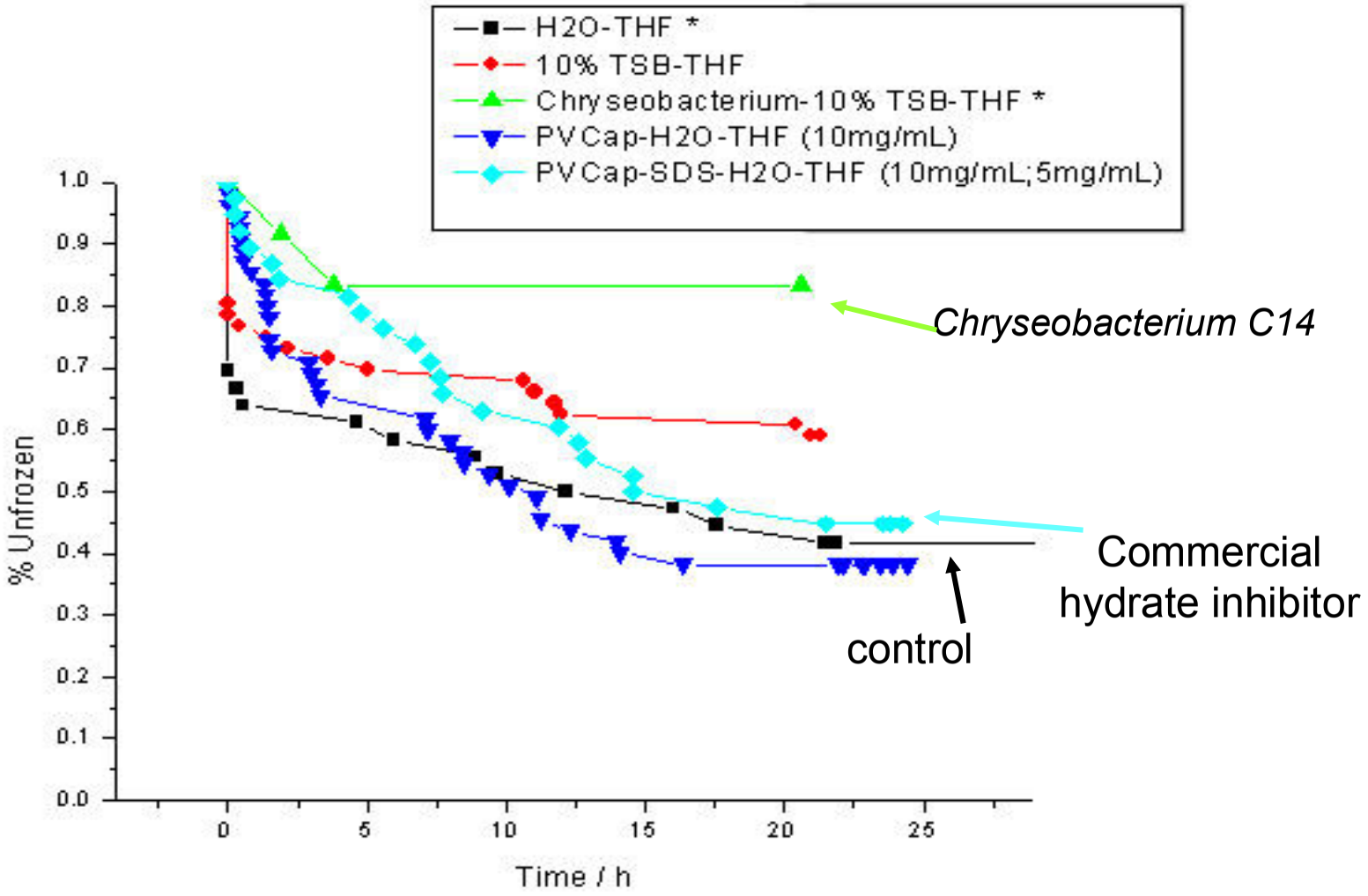
Hydrates recrystallize faster after being melted

BUT this fast recrystallization is inhibited with active AFPs!



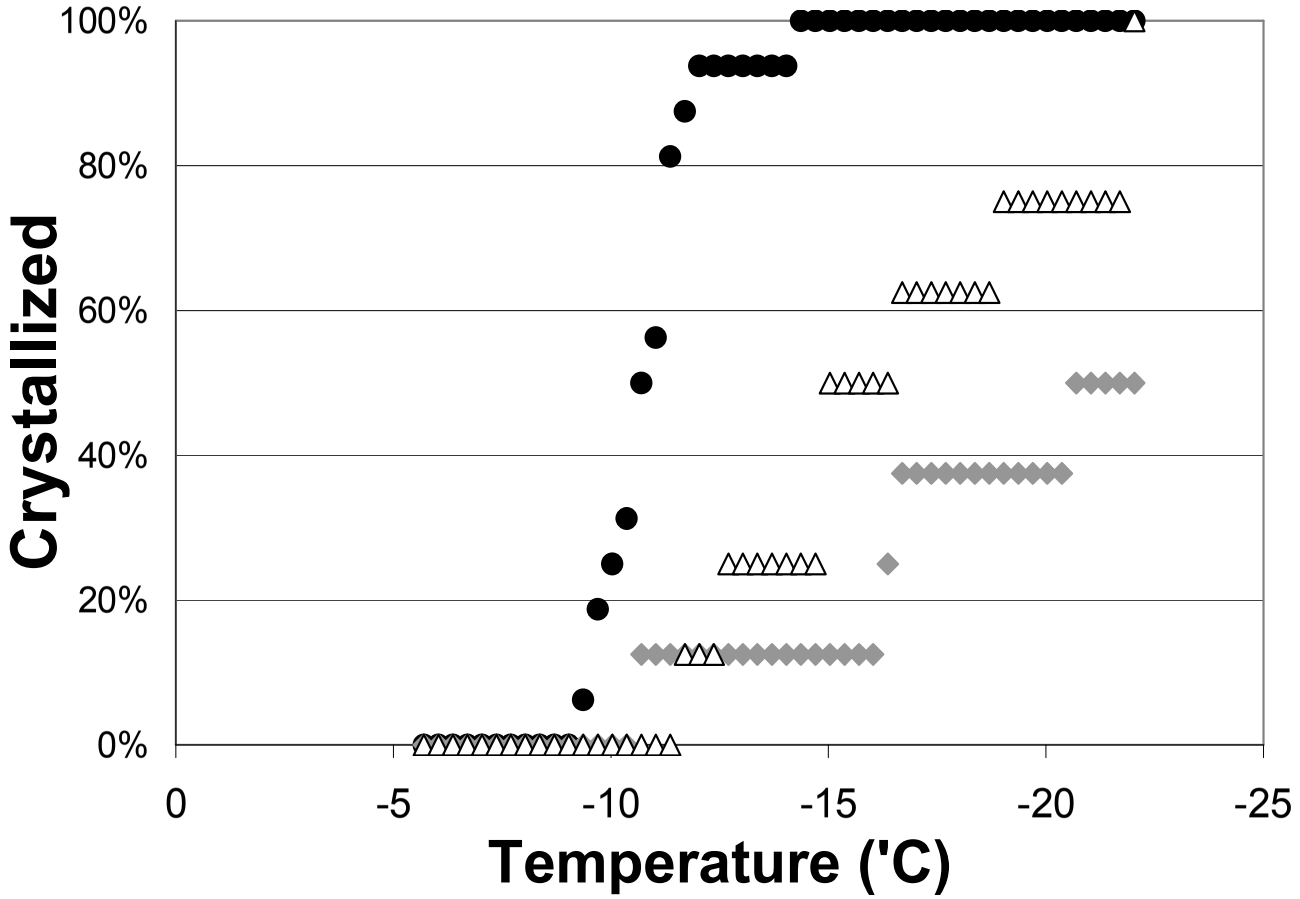
Sound familiar?

What about the backyard microbes? Can they also inhibit hydrate formation?



What about the backyard microbes? Can they also promote hydrate formation?

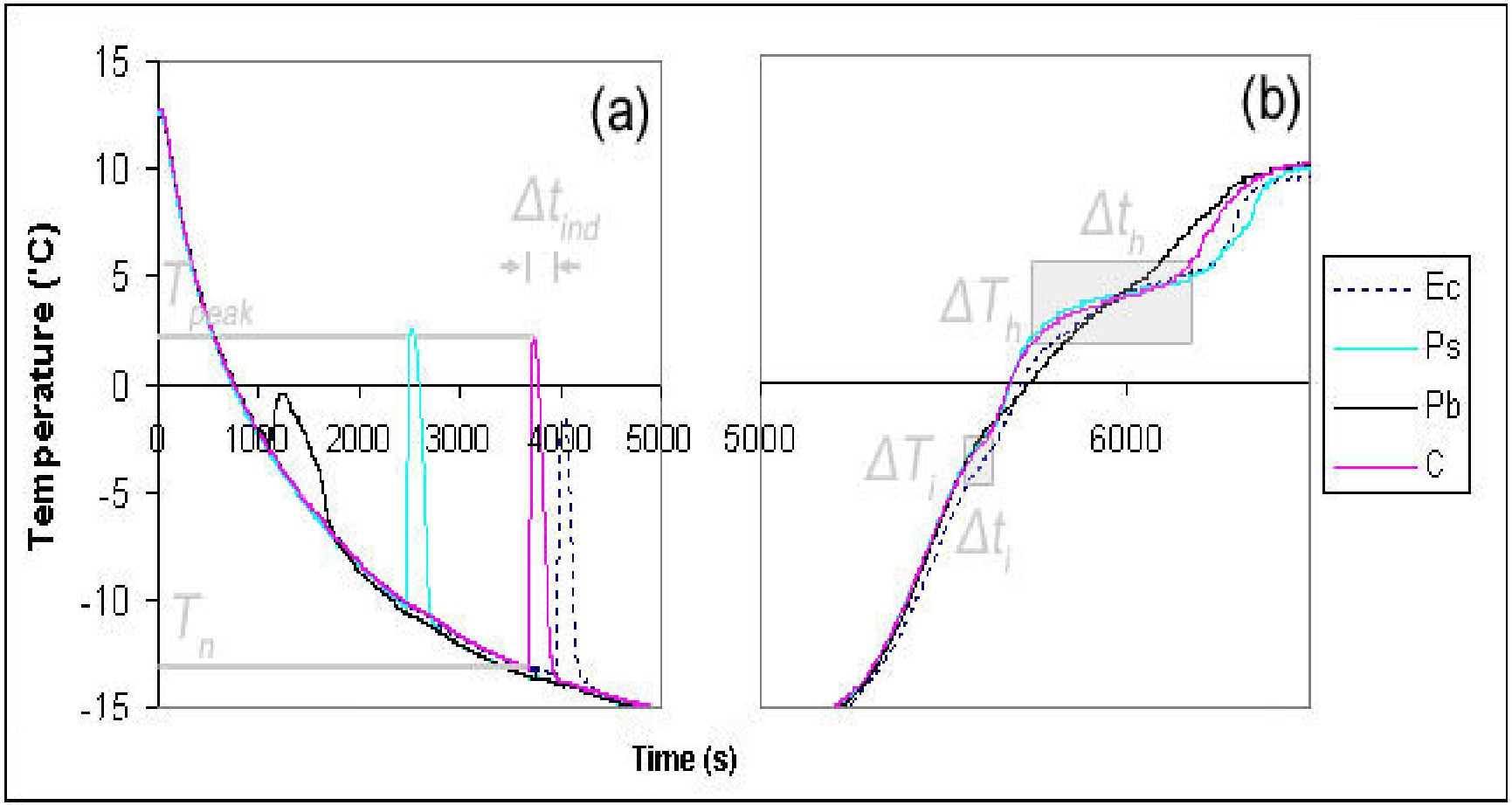
Crystallization spectrum of a model hydrate



- *P. borealis*
- Δ control solutions
- ◇ control cultures

So, as well as inhibiting hydrate formation, can this property be used as a kind of anti-agglomerate to prevent hydrate blockage? Or for hydrate transport?

Emily Huva's work: Thermodynamics of freezing THF hydrate in the presence of microbes



Experimental uncertainty is ± 0.5˚C.

What microbes would we find on Mars? a comet? a hydrate Popsicle? <yuk!>

Question: would a hydrate Popsicle model conditions 'out there'?

Resistance is not futile- it allows survival

Microbes here on earth show us just how resilient microbial populations can be – so is there life out there?