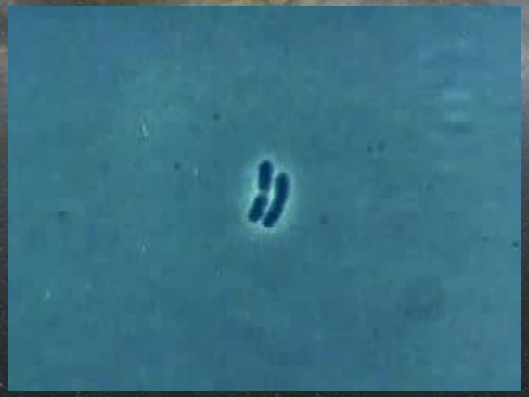
COULD MICROORGANISMS IN PERMAFROST HOLD THE SECRET OF ETERNAL LIFE?

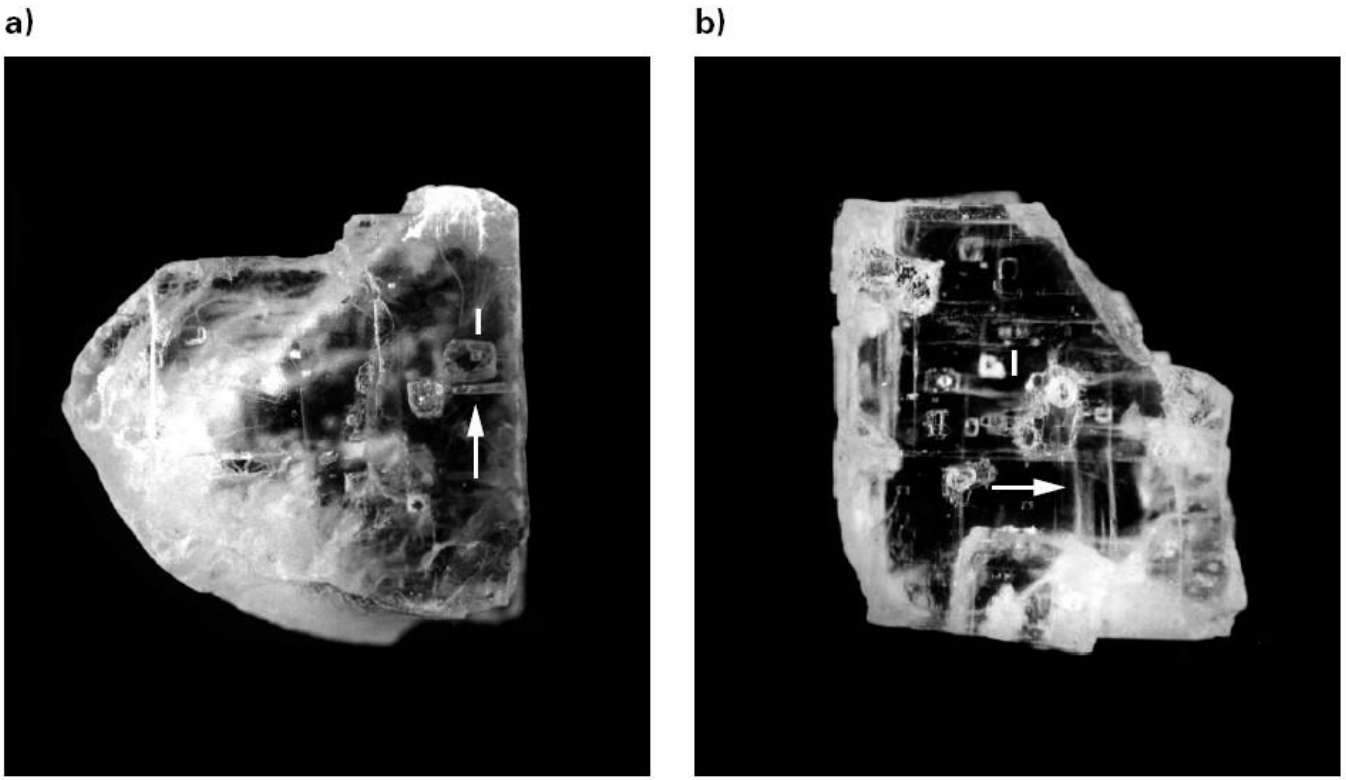
A.Brouchkov,V.Melnikov G.Griva and V. Repin



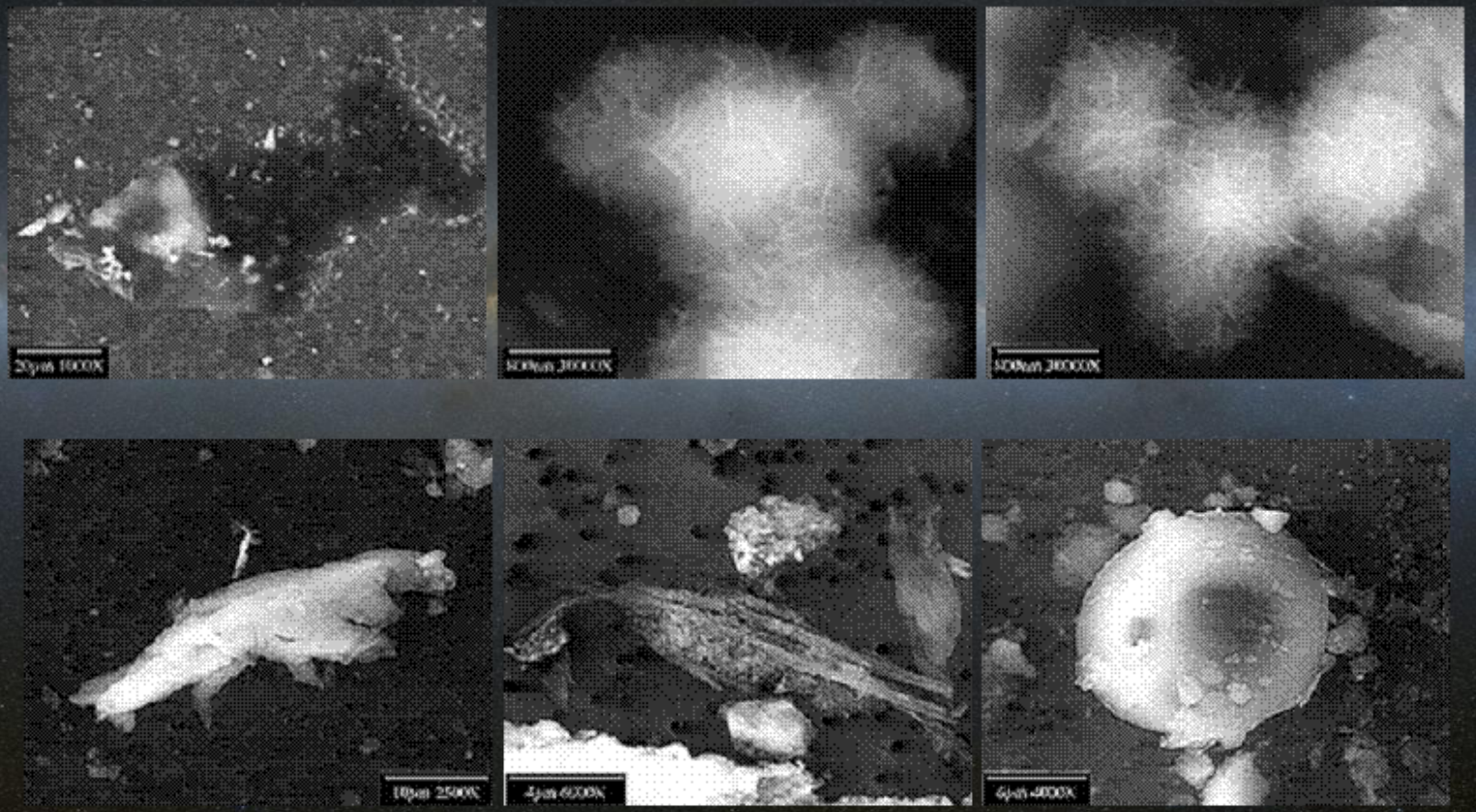
How old can be a single cell?

Bacteria Survives in 250-Million-Year-Old Salt Crystal

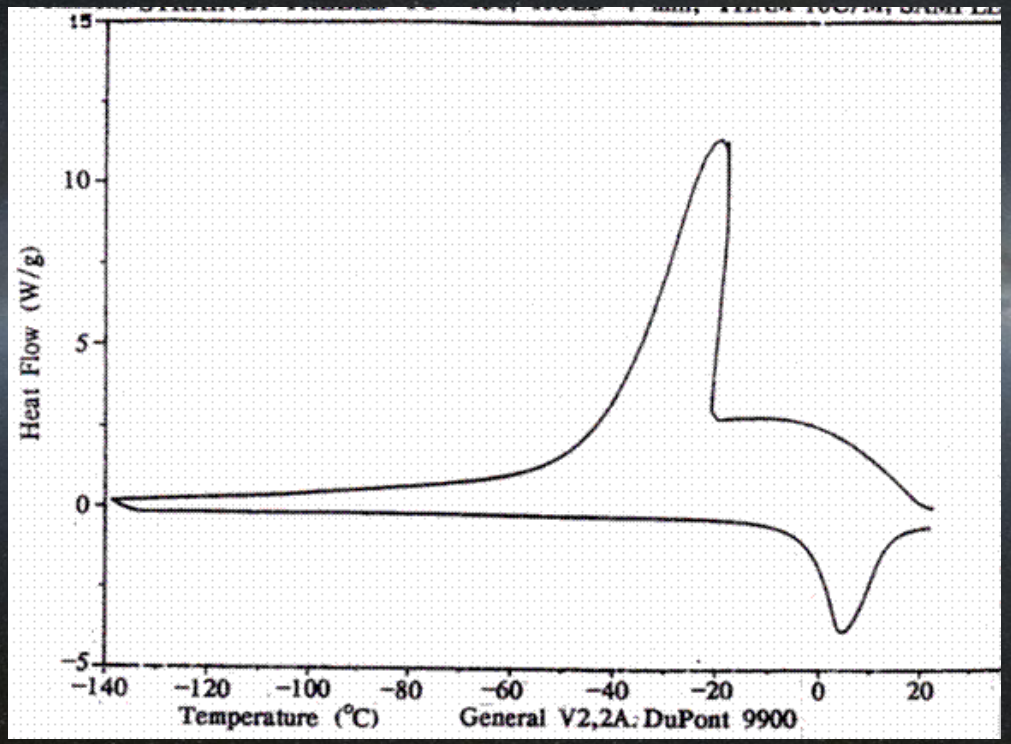
FIGURE 2.18 • Salt crystals from 1,850 feet down an air intake shaft in Carlsbad, New Mexico. **a)** This crystal appeared undisturbed due to the clarity and shape of the fluid-filled chamber. This crystal contained the strain 2-9-3. The drill hole used to obtain the sample (shown above the arrow) permitted access to the inclusion (I), or chamber, containing the bacteria. **b)** This crystal was rejected since it contained cracks (arrow points to a large vertical crack) and the inclusion (I) is irregular in shape.



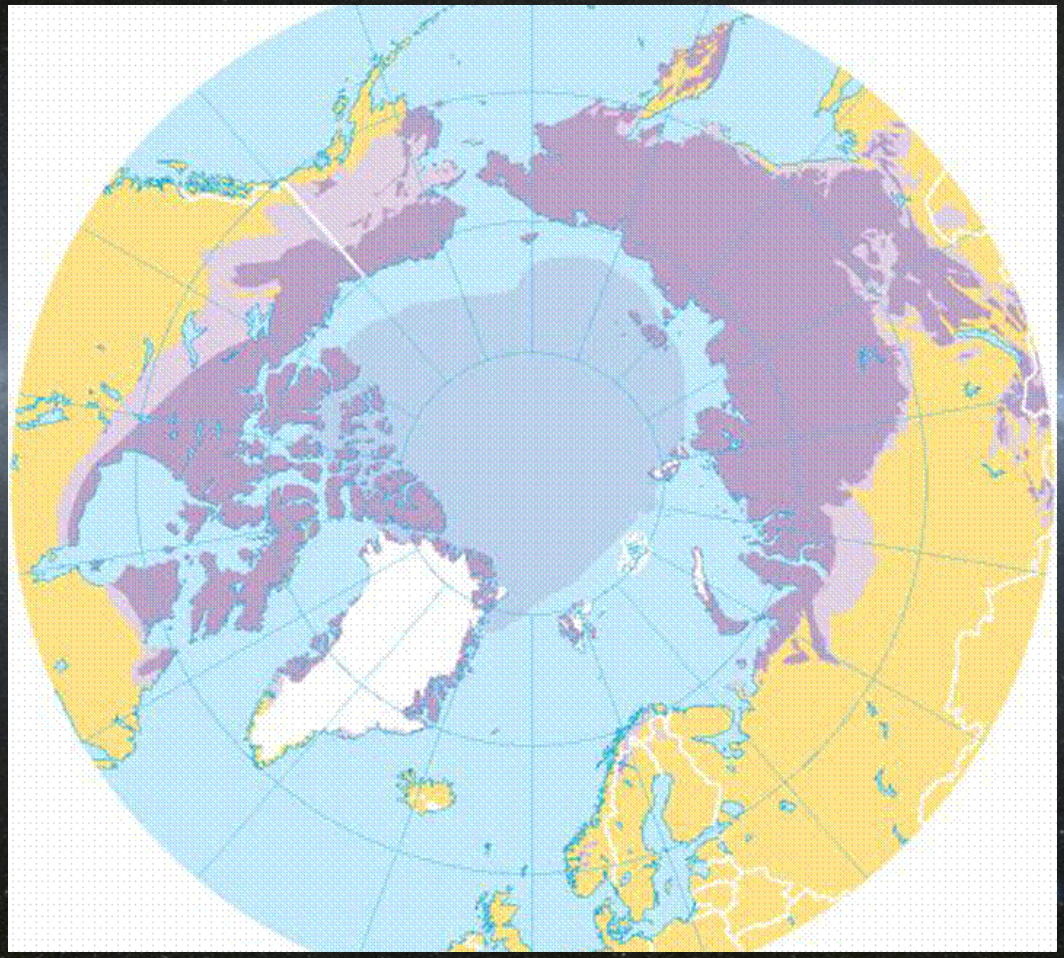
Live 500,000 year-old microbial cells were found in Antarctic ice by scientists from the Institute of Microbiology in Moscow almost 30 years ago



Permafrost temperature are not low enough (-3 to -7 degrees C) to freeze trapped bacterial cells



Freezing of bacterial culture, from McGrath, Wagener and Gilichinsky, 1994

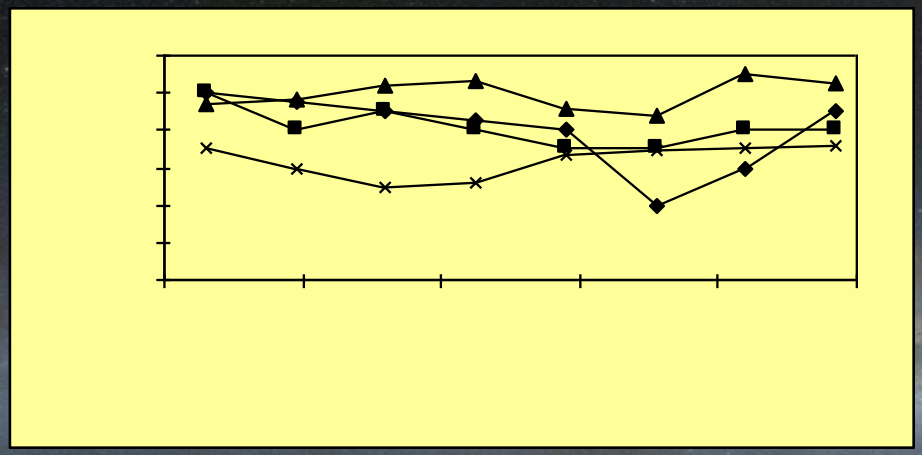


Area of permafrost in the Northern Hemisphere

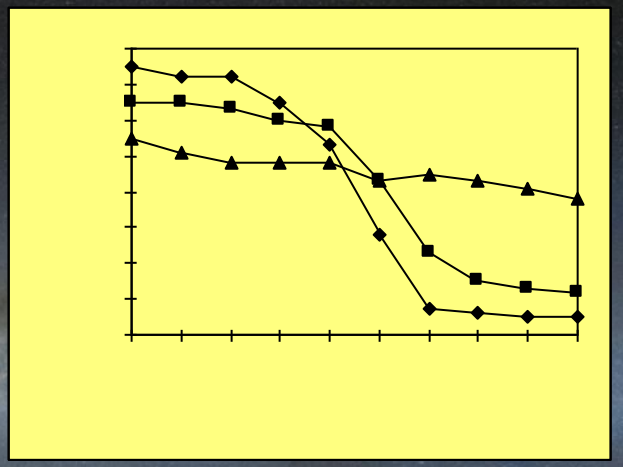
Permafrost

Structure of permafrost is stable – nothing to change for thousands and millions of years

Water and salts movement inside of permafrost

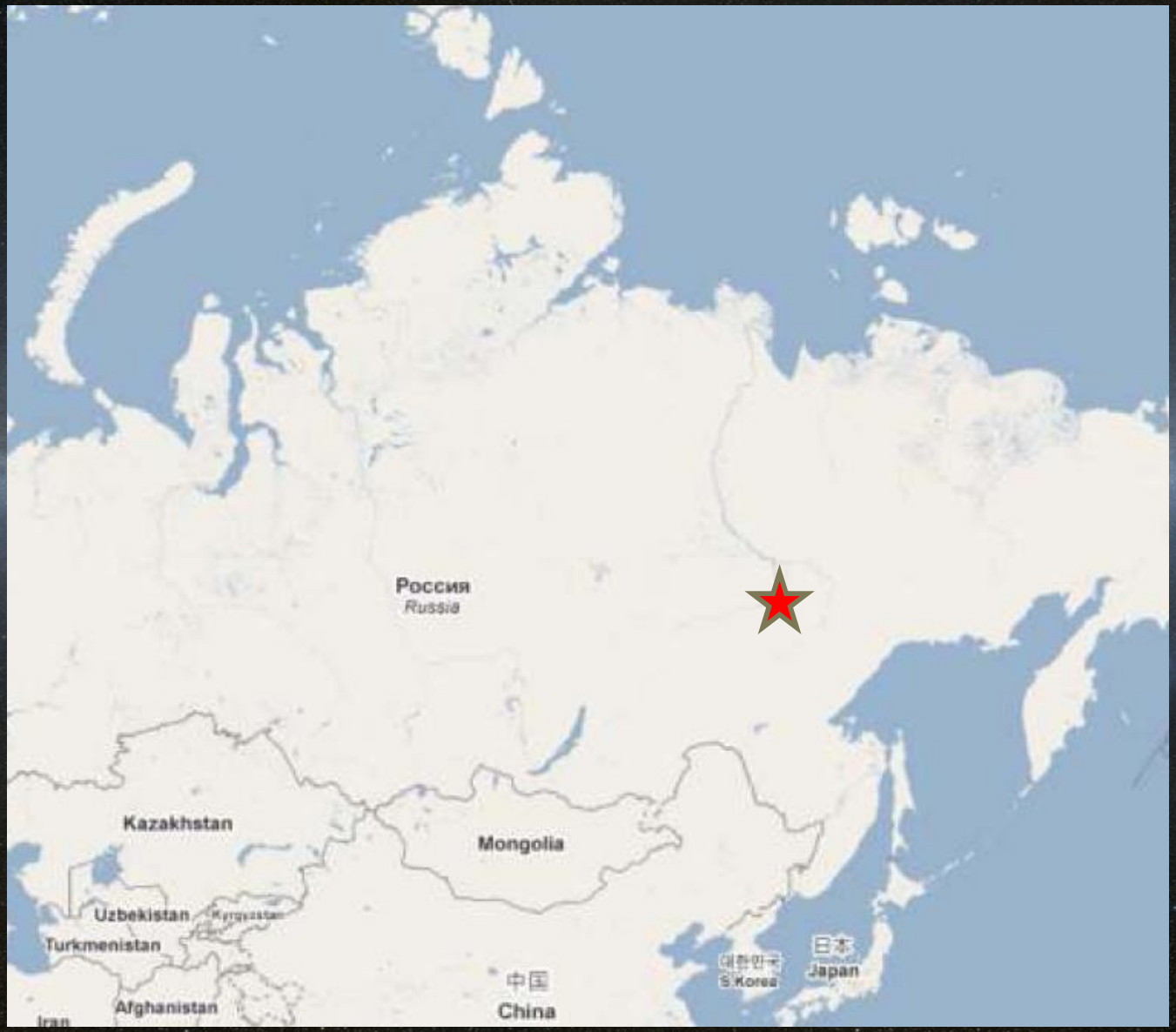


Distribution of water content, W, % in frozen marine silt under the influence of a temperature gradient (at the right «warm» side -2.2°C, on the left side -2.7°C): 1 - initial; 2 – after 1 year; 3 - after 5 years; 4 - after 11 years



Sea salt transfer in marine silt at temperature -3°C: 1 - an initial distribution of salinization; 2 - after 7 months, 3 - after 11 years of experiment

Sampling in Siberia



Ice wedges in Siberia

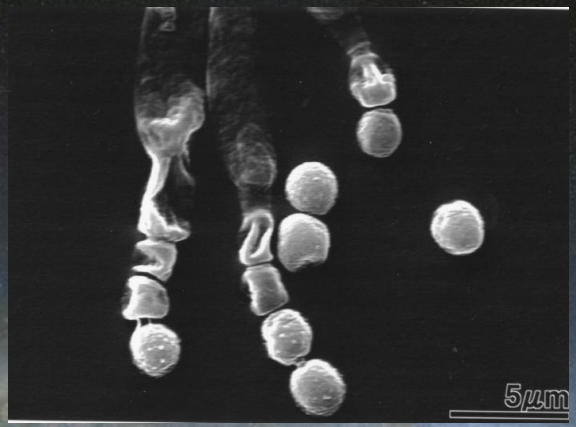
Aldan river exposure, about 40 m above water level (left) and Sirdah lake exposure, about 10 m above water level (right). Ice wedges do not contain as much methane as frozen grounds, but the average content is high. Icewedges are different: Sirdah site dosn't contain methane, but carbon dioxide; Neleger site contain much methane.

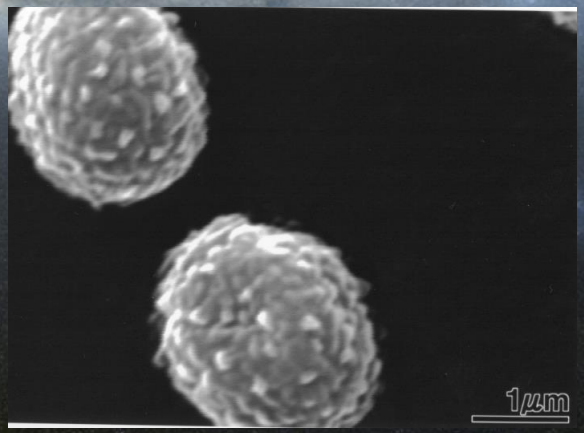
Permafrost sampling techniques to avoid contamination

Underground laboratory in Yakutsk

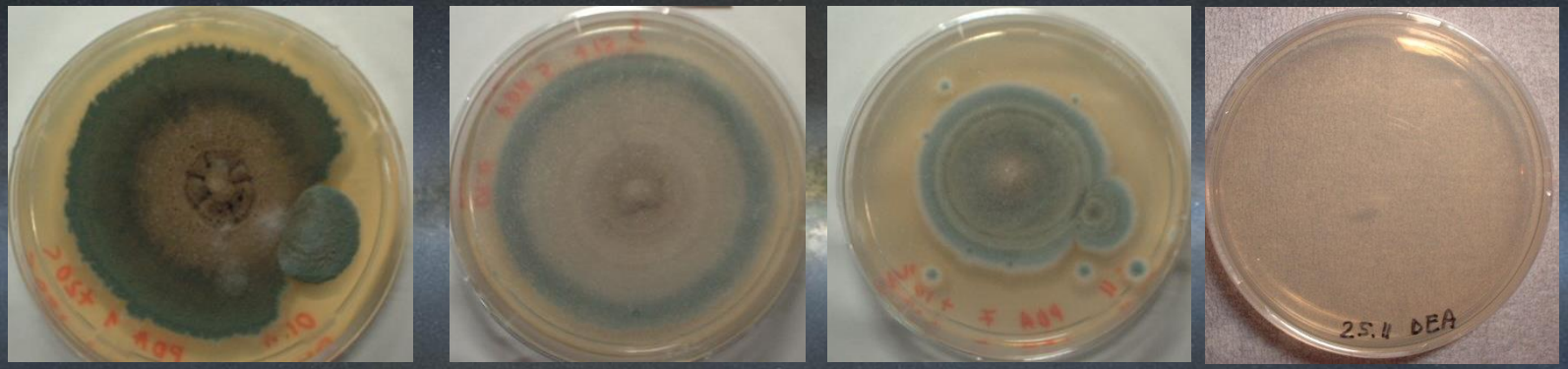
Spores of *Penicillium sp.* found in permafrost

(Dr. M. Tanaka pictures)



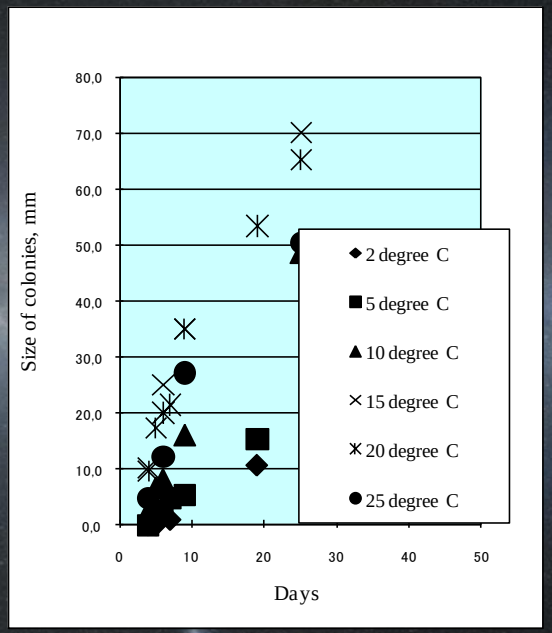


Fungi growth at different temperatures



Growth of Penicillium echinulatum, PF strain from underground laboratory at different temperatures: a - 20°C; b - 15°C; c -10°C; 25 days of incubation; d - -5°C; 2 months of incubation

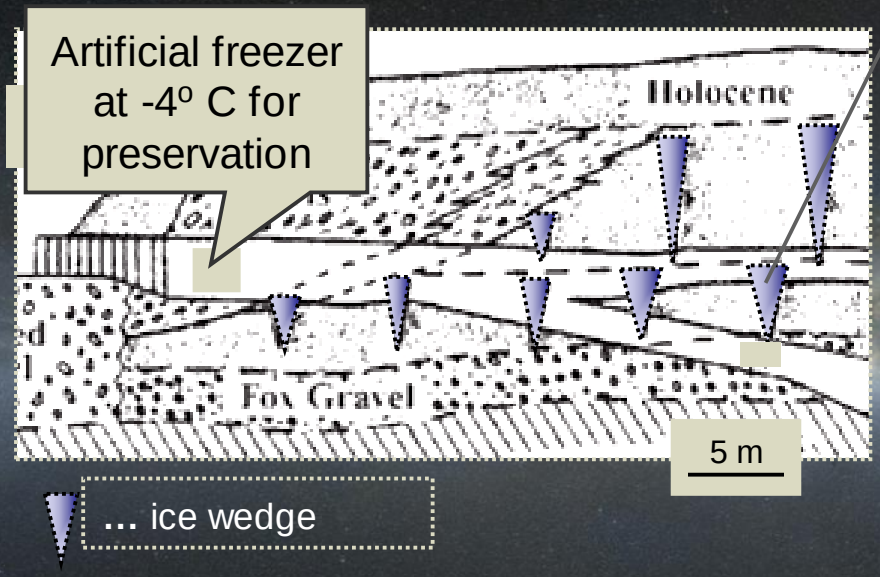
Fungi growing at low temperatures below zero degree C



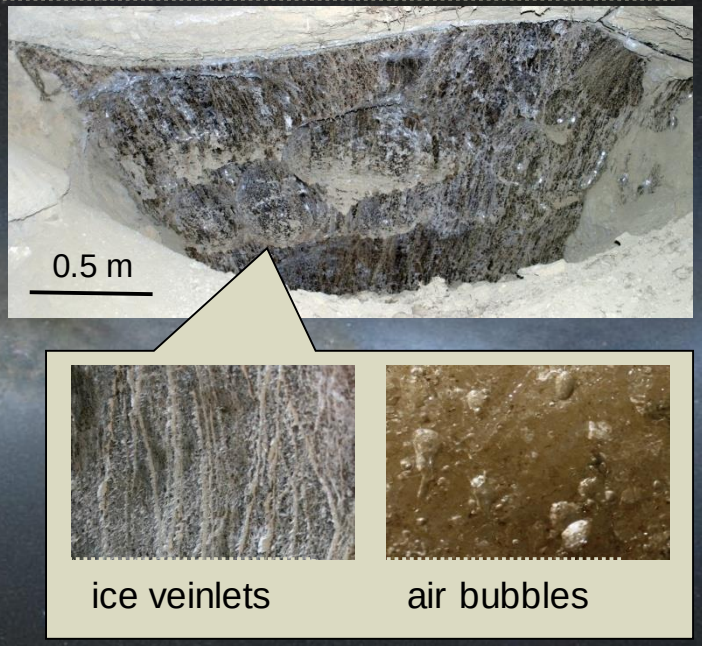
Ice wedge in Fox Permafrost Tunnel, Alaska

(work done by Dr. M.Tanaka and T.Katayama)

The Fox Permafrost Tunnel is located near Fairbanks in central Alaska.



exposed part of buried ice wedge



- The shape and internal fabrics of the ice wedges in this tunnel show no signs of thawing.
  - continuously frozen
  - closed environment

Material and Methods

State of Alaska, U.S.A

Fox Permafrost Tunnel

sample collection

transfer

Radio-carbon Dating

result; 24,884±139 yr BP

continuously frozen for 25,000 years

Surface-sterilization (at -5° C)

70% ethanol — Flame

Melted ice

inoculum — direct plating

15° C

aerobically

Isolation

colony selection and restreaking

(filter-sterilized; ice extract)

Component of media

Supernatant

Precipitate

DNA extraction

16S rDNA clone library

Partial 16S rDNA sequence analysis

(*E. coli* position 27 to 520)

Medium constituent

- Luria-Bertani agar (LB)
- LB+1.0% of Glucose (LBG)
- R2B
- 100 times diluted LB (1/100 LB)
- 1/100 LBG
- Minimal Medium (MM)
- MM+0.5% of Glucose (MMG)
- MM+1.0% of Ice extract (MME-1)
- MM+10% of Ice extract (MME-2)
- Hickey-tresner diluted agar medium with antibiotics (H.T.D.A)

R2B (per liter)	
peptone	2.0 g
Yeast extract	2.0 g
Casamino Acids	2.0 g
Glucose	2.0 g
Soluble starch	2.0 g
Sodium pyruvate	1.2 g
K_2HPO_4	1.2 g
$MgSO_4 \cdot 7H_2O$	0.2 g

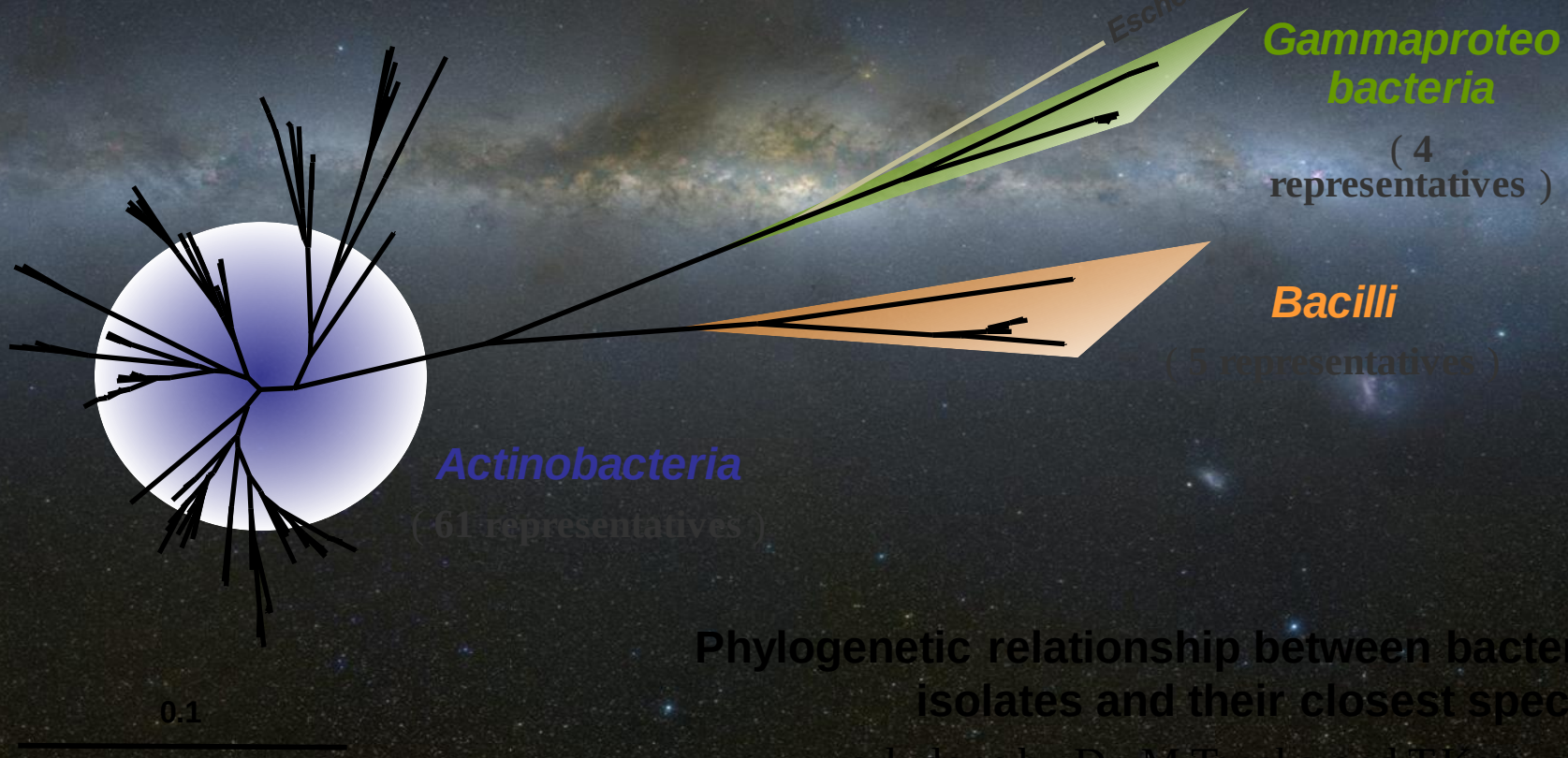
H.T.D.A (per liter)	
peptone	0.4 g
Yeast extract	0.2 g
Meat extract	0.2 g
Soluble starch	2.0 g
Nystatin	50 ppm
Cycloheximide	100 ppm
Nalidixic acid	5 ppm

Minimal Medium (per liter)	
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	200 mg
$FeSO_4 \cdot 7H_2O$	10 mg
$CaCl_2 \cdot 2H_2O$	10 mg
NH_4Cl	1.0 g
Trace elements	0.1 mg

All plates contained 2.0% of agar and were adjusted at pH 7.0

Phylogenetic analysis of aerobic isolates

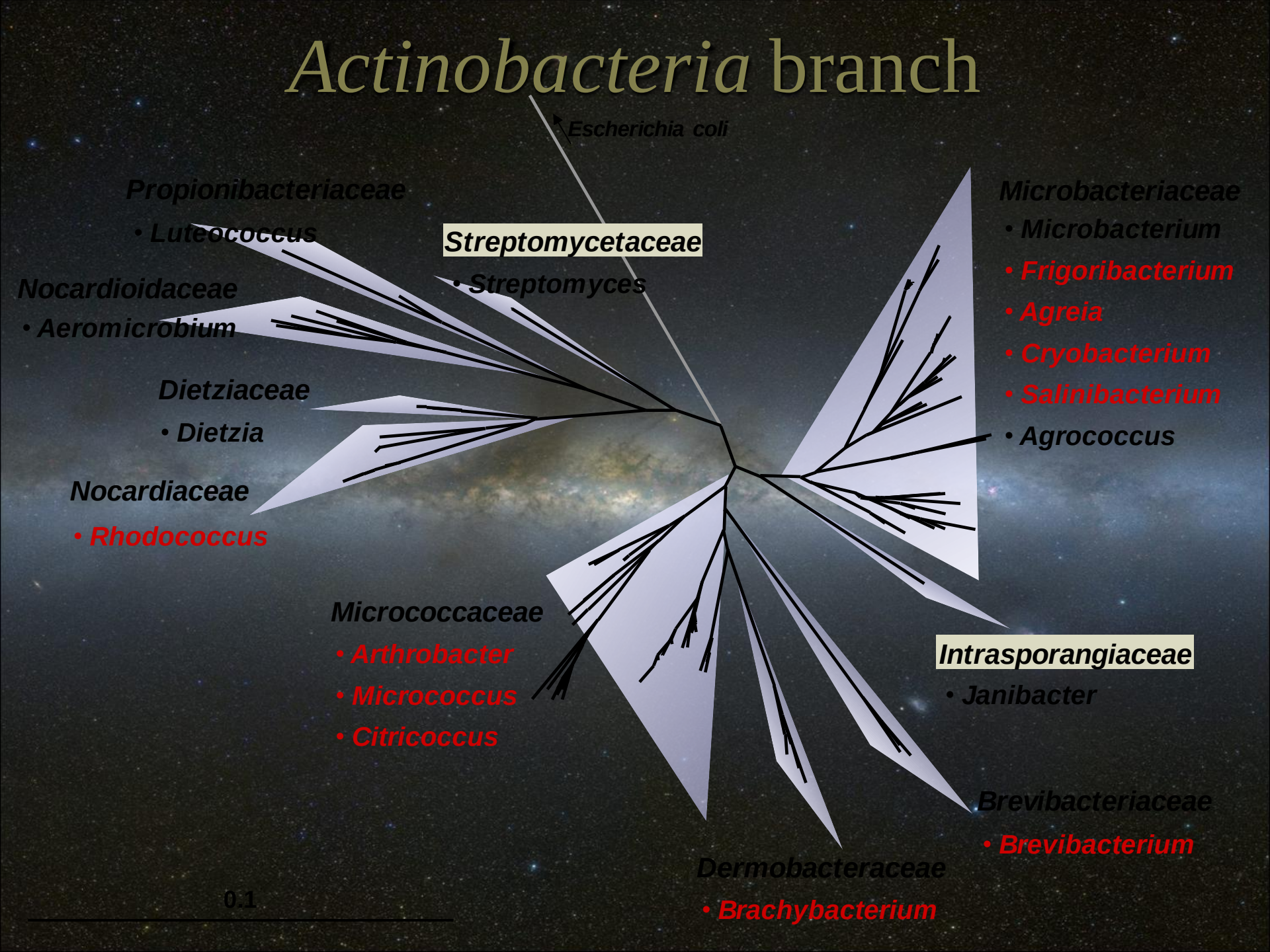
- 10^3 to 10^6 CFU / ml of melted ice
- **301** strains were isolated
- **70** representative strains by partial 16S rDNA sequences



Phylogenetic relationship between bacterial isolates and their closest species

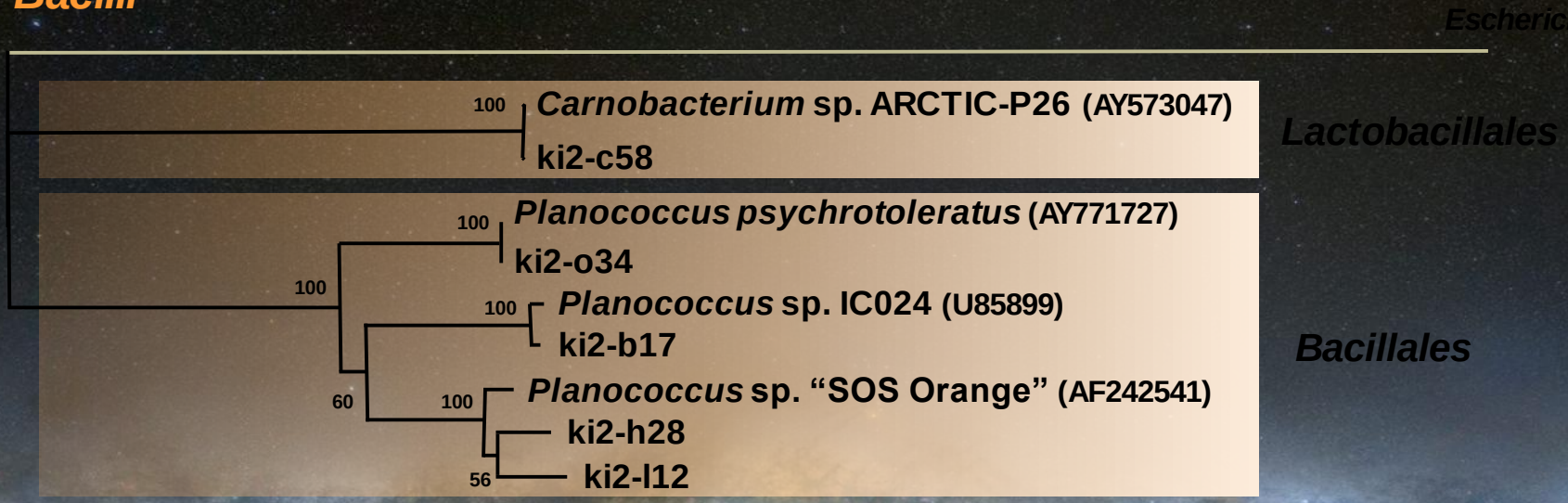
work done by Dr. M.Tanaka and T.Katayama

Actinobacteria branch

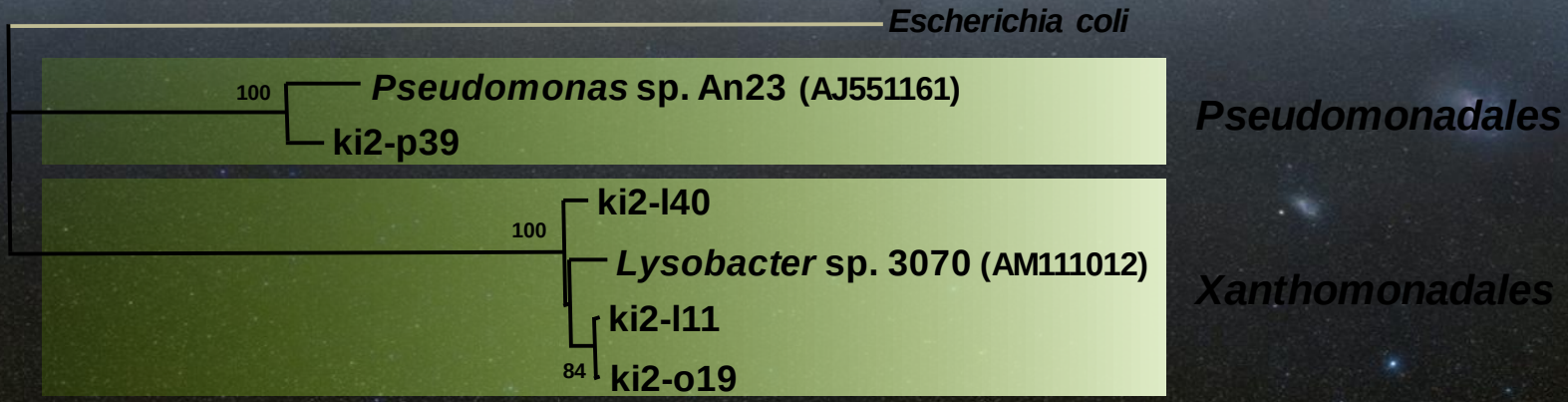


Firmicutes and Gamma-*Proteobacteria* branches

- ***Bacilli***



- ***Gammaproteobacteria***



work done by Dr. M.Tanaka and T.Katayama

New species ?

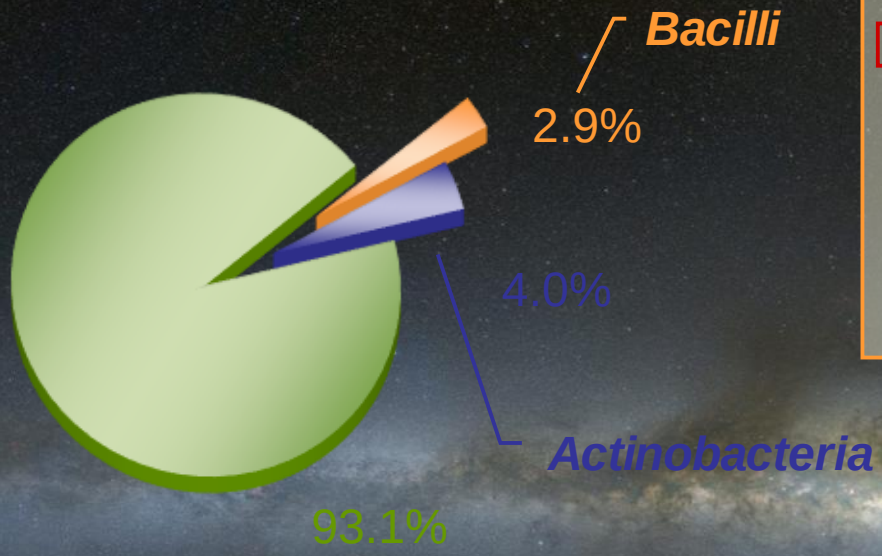
- less than 97% similarity with those in database (partial 16S rDNA sequences)

No.	Closest species (Accession no.)	Similarity
ki2-o11	*Arthrobacter ramosus* (AM039435)	96.5%
ki2-j34	*Arthrobacter woluwensis* (X93353)	96.8%
ki2-m9	*Agrococcus jenensis* (AJ717350)	95.5%
ki2-j21	*Agreia* sp. 37-4 (AF513393)	96.7%
ki2-j22	*Frigoribacterium* sp. GWS-SE-H243 (AY332185)	96.9%
ki2-h51	*Salinibacterium* sp. 7320 (AM111058)	95.4%
ki2-j18	*Frigoribacterium* aff. faeni A-1/C-an/E (AJ297441)	96.5%
ki2-m15	*Curtobacterium* sp. B20 (AF128869)	94.6%
ki2-h47	*Brevibacterium* sp. SK8E11 (DQ153944)	96.2%
ki2-m17	*Rhodococcus fascians* (AJ011329)	95.7%
ki2-g6	*Rhodococcus* sp. P27-27 (DQ060384)	95.5%
ki2-l37	*Luteococcus peritonei* (AJ132334)	94.8%
ki2-l20	*Nocardioides jensenii* (AF005006)	96.4%
ki2-j4	*Aeromicrobium* sp. Gsoil 098 (AB245394)	95.9%
ki2-l2	*Aeromicrobium panaciterrae* (AB245387)	96.7%

work done by Dr. M.Tanaka and T.Katayama

Phylogenetic analysis of clones

- 273 clones were analyzed



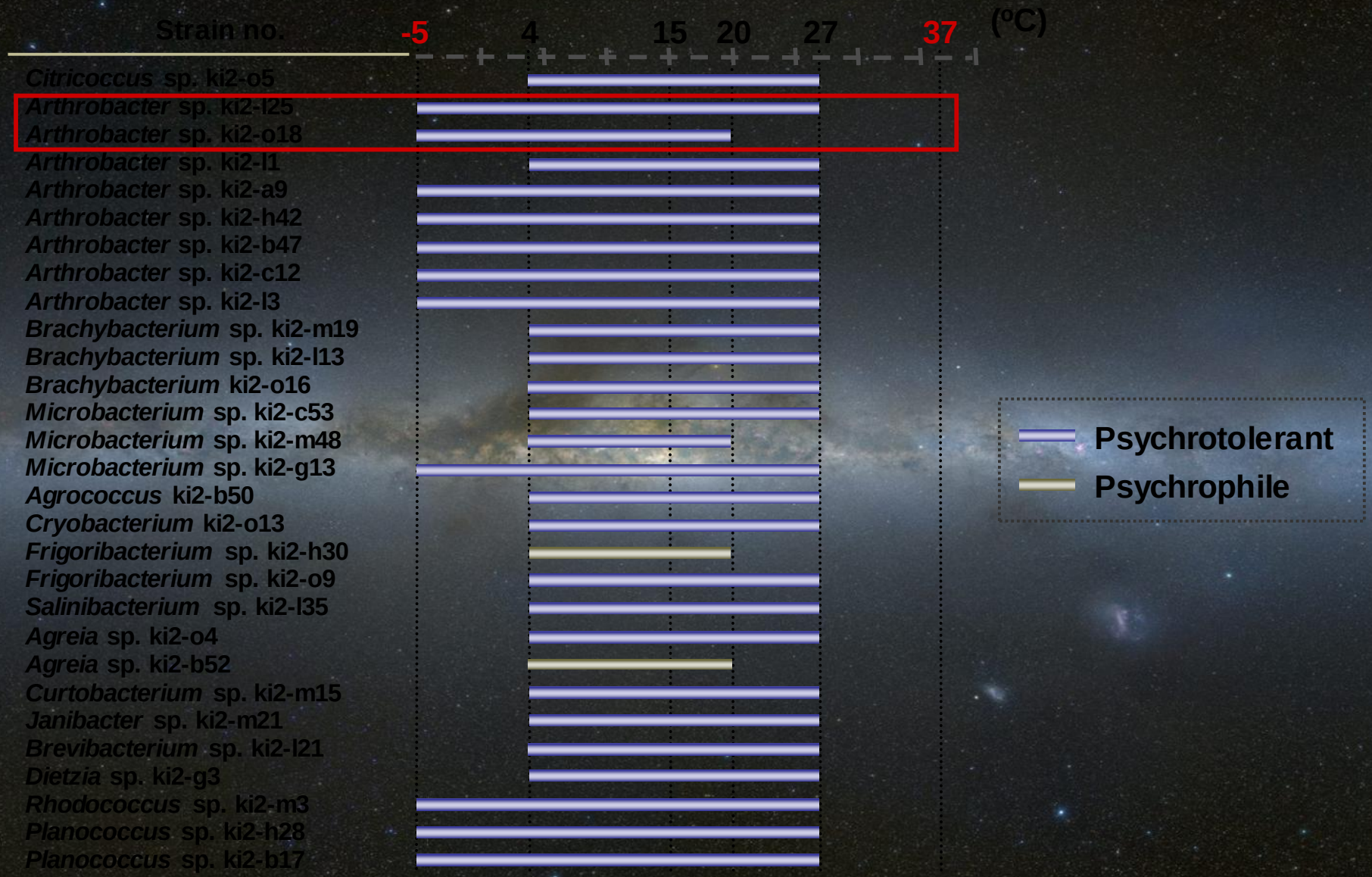
Type	Closest species (Accession no.)	Number
t23	*Carnobacterium* sp. ARCTIC-P26 (AY573047)	1
t86		1
s124	*Planococcus* sp. IC024 (U85899)	1
s96		2
s42		1
t9		1
t38		1

Type	Closest species (Accession no.)	Number
t32	*Cryobacterium psychrophilum* (AY526664)	1
s84	*Agreia* sp. 37-4 (AF513393)	1
s135	*Arthrobacter* sp. An26 (AJ551164)	2
s173	*Arthrobacter* sp. 45-3 (Ay444853)	1
s37	*Arthrobacter stackebrandtii* (AJ640198)	1
t85	*Arthrobacter* UVvi (AY220354)	1
s142	*Arthrobacter* sp. 130-8 (AY444862)	2
s13		1
t27		1

Type	Closest species (Accession no.)	Number
s106	*Pseudomonas* sp. NZ111 (AY014825)	1
s113	*Pseudomonas syringae* (AY275478)	1
s151	*Pseudomonas* sp. E-3 (AB041885)	1
s47	*Pseudomonas* sp. P1 (AY568577)	1
s206	*Pseudomonas* sp. An23 (AJ551161)	161
s201		16
t98		72
s193		1

Distribution of partial 16S rDNA clones

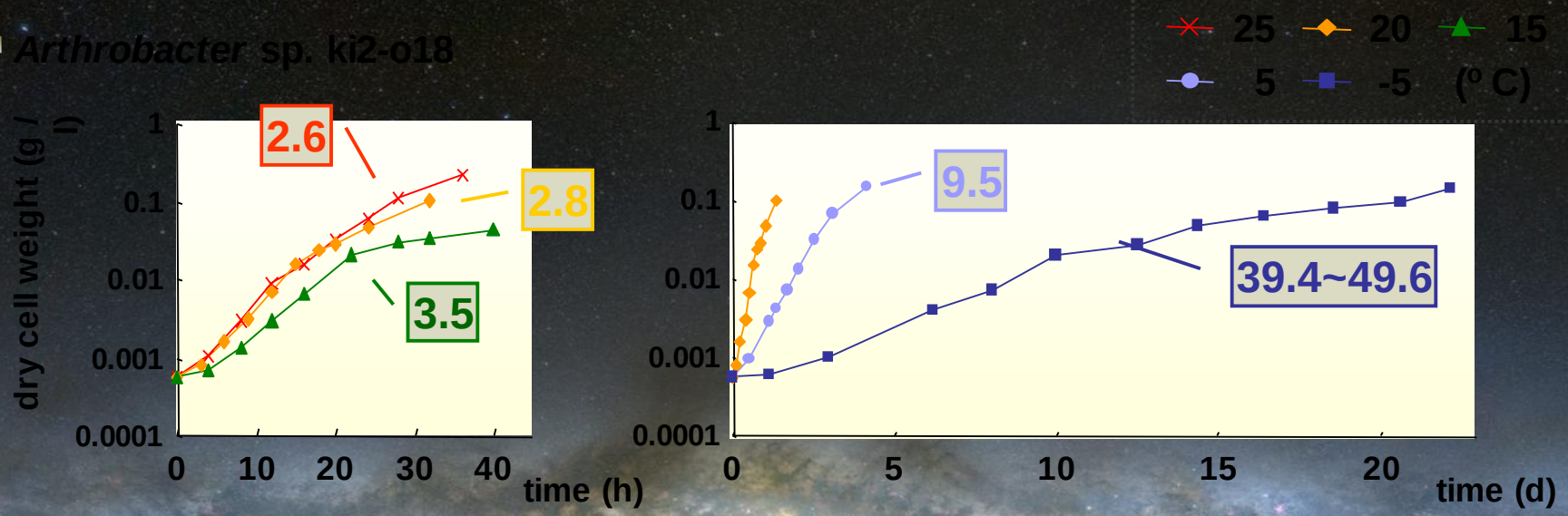
Temperature sensitivity of bacterial isolates



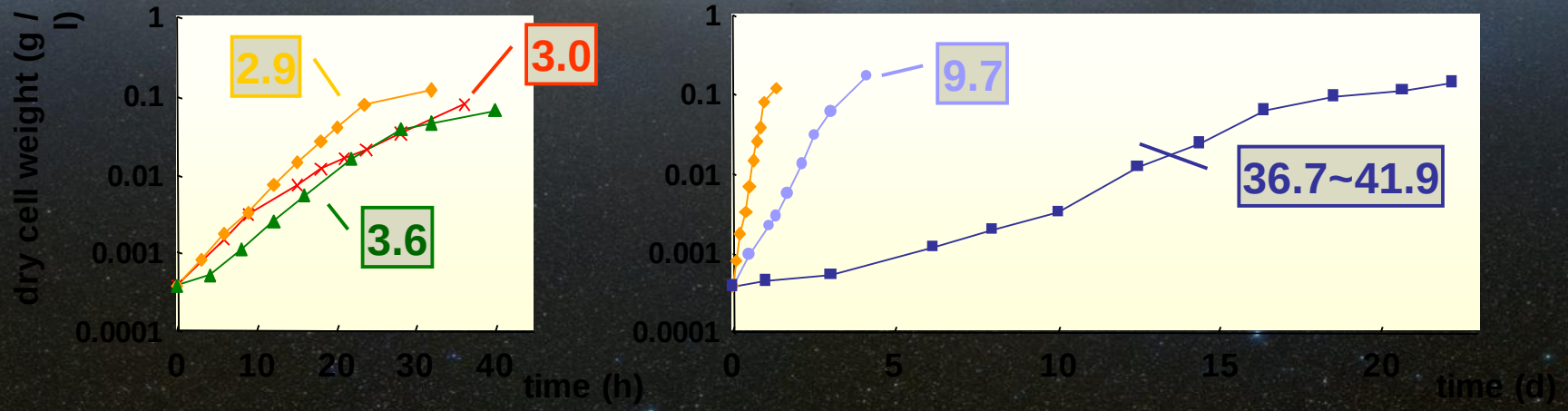
Growth were checked by colonial diameter in LB agar medium for 5 days to 3 months.

The doubling times of *Arthrobacter* spp.

- ***Arthrobacter* sp. ki2-o18**



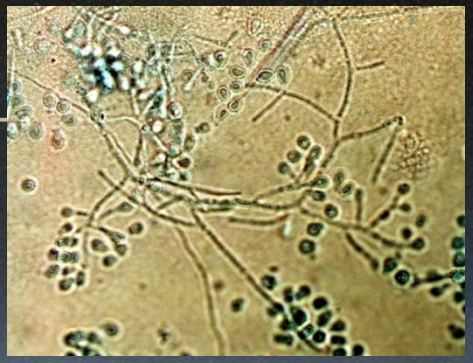
- ***Arthrobacter* sp. ki2-l25**



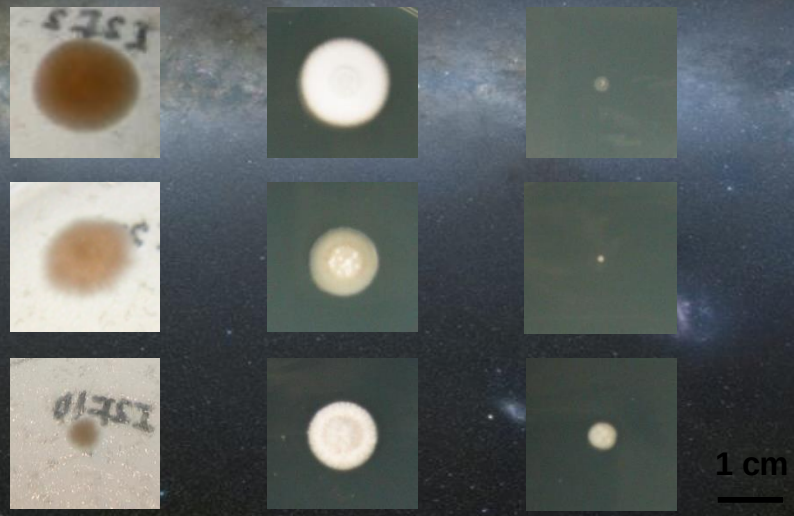
shaking incubation at 150 rpm^{-1} in LB medium

Fungal isolates and their sensitivity to temperature

Identification of isolated fungi and their colonies at -5, 20 and 27 ° C on potato dextrose agar medium



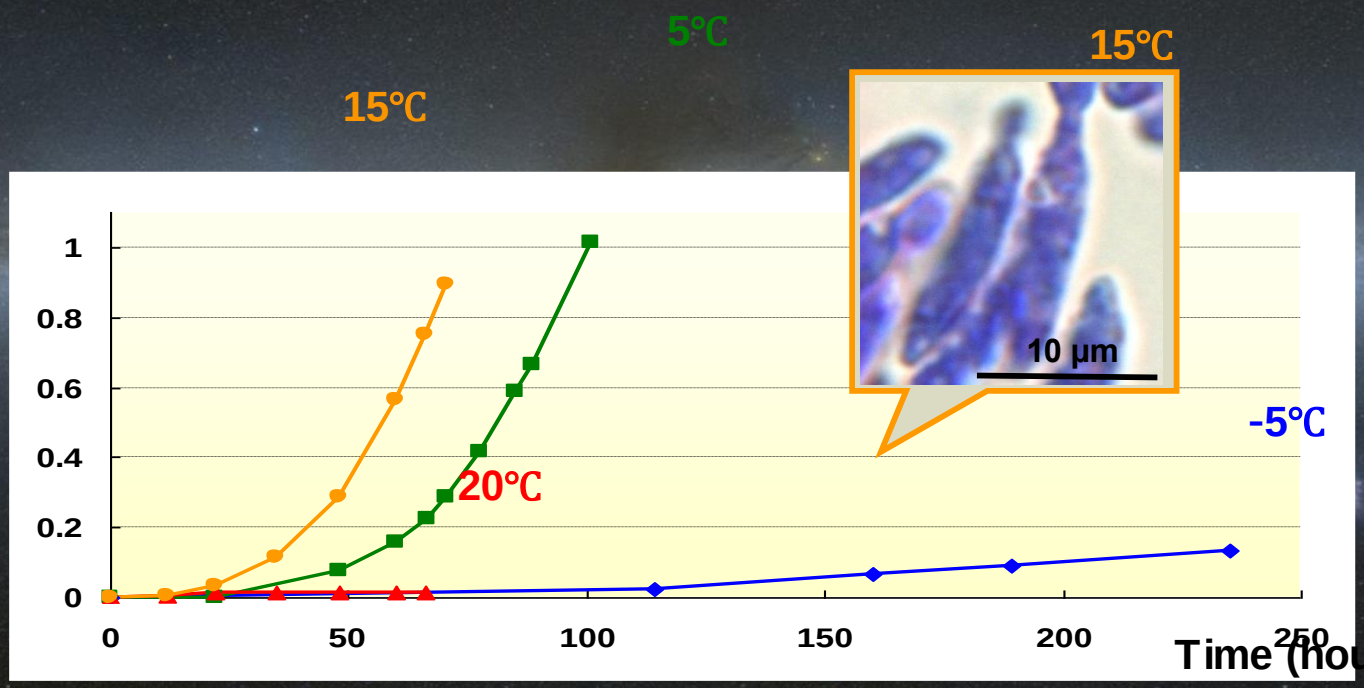
Strain	Closest species (Accession no.) (Similarity)
I2Fp75	*Phaeococcomyces nigricans* (AJ276065) (99.0%)
I2F7	*Leucosporidium antarcticum* (AF44529) (100%)
I2F2	*Geomyces* sp. FFI 30 (AJ608960) (99.3%)
I2F3	*Geomyces* sp. FFI 30 (AJ608960) (99.2%)
I2F10	*Geomyces* sp. FFI 30 (AJ608960) (99.8%)



-5° C	20° C	27° C
(3 months)	(7 days)	(7 days)

work done by Dr. M.Tanaka and T.Katayama

The growth of isolated yeast



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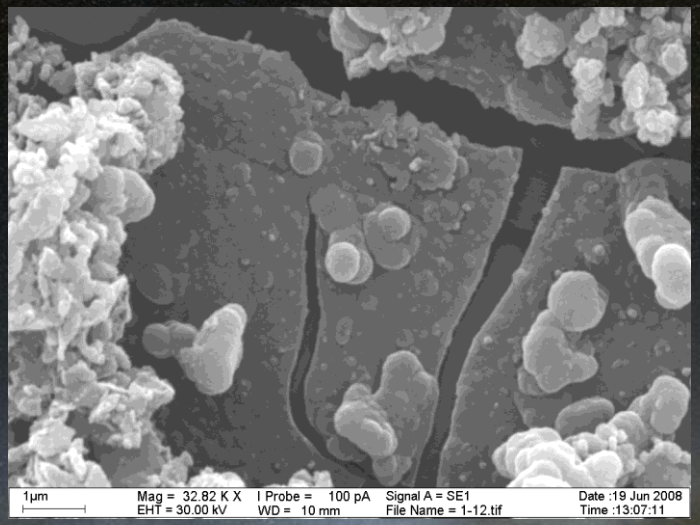
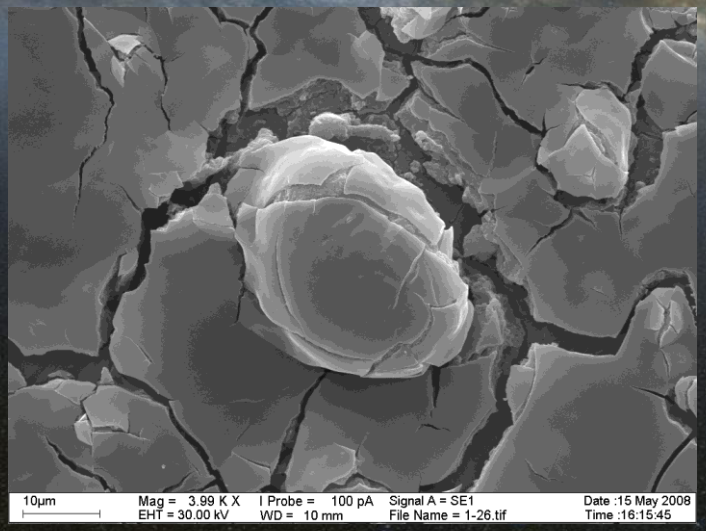
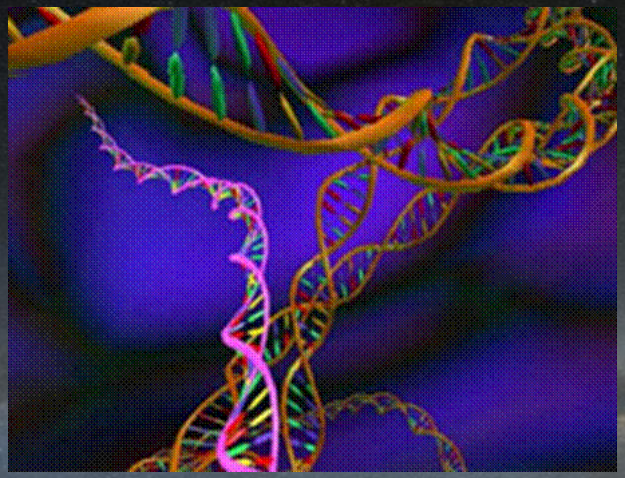




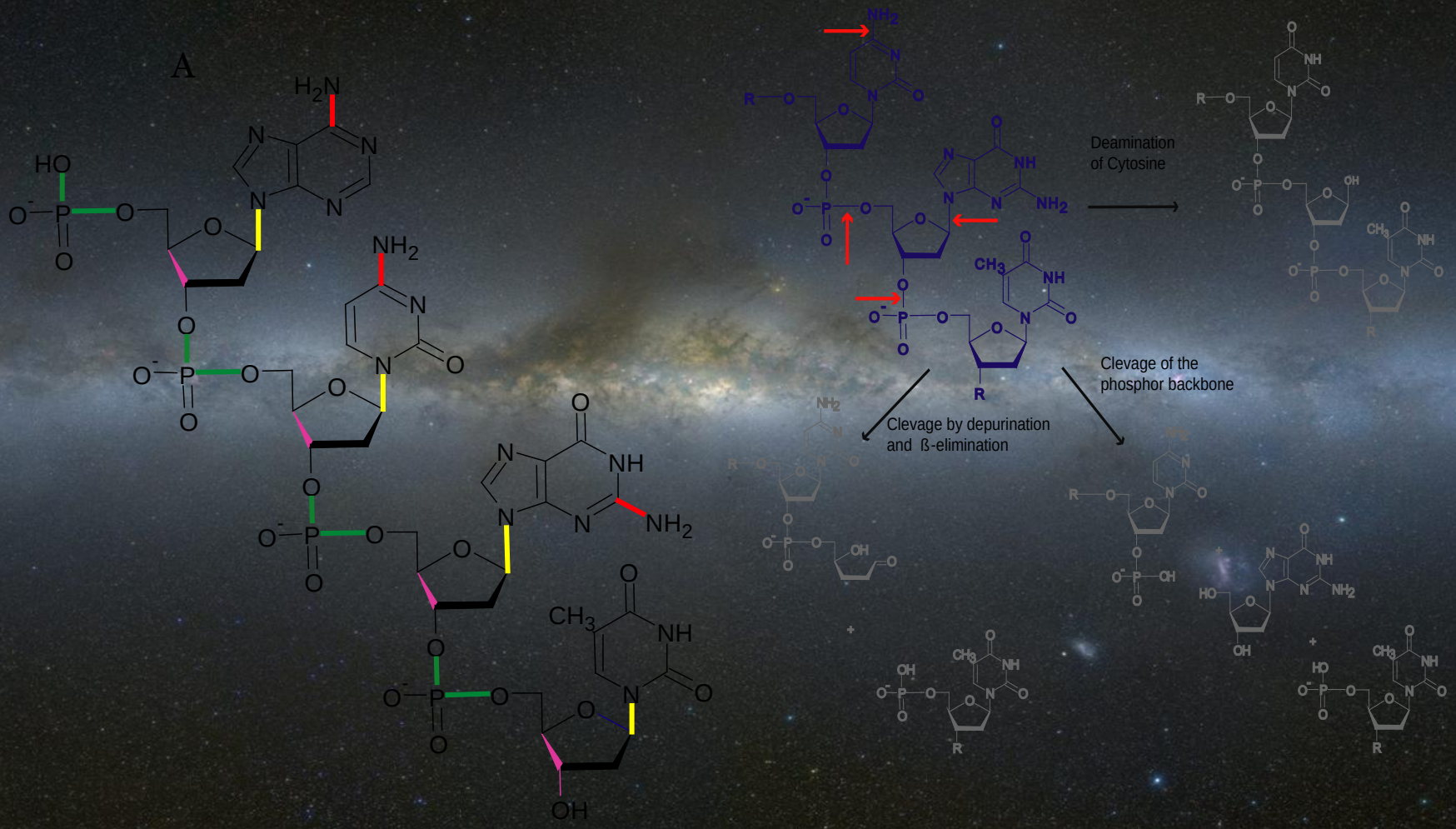
Ice from Mammoth Mountain aged about 40000 years

Why are the bacterial cells still alive?



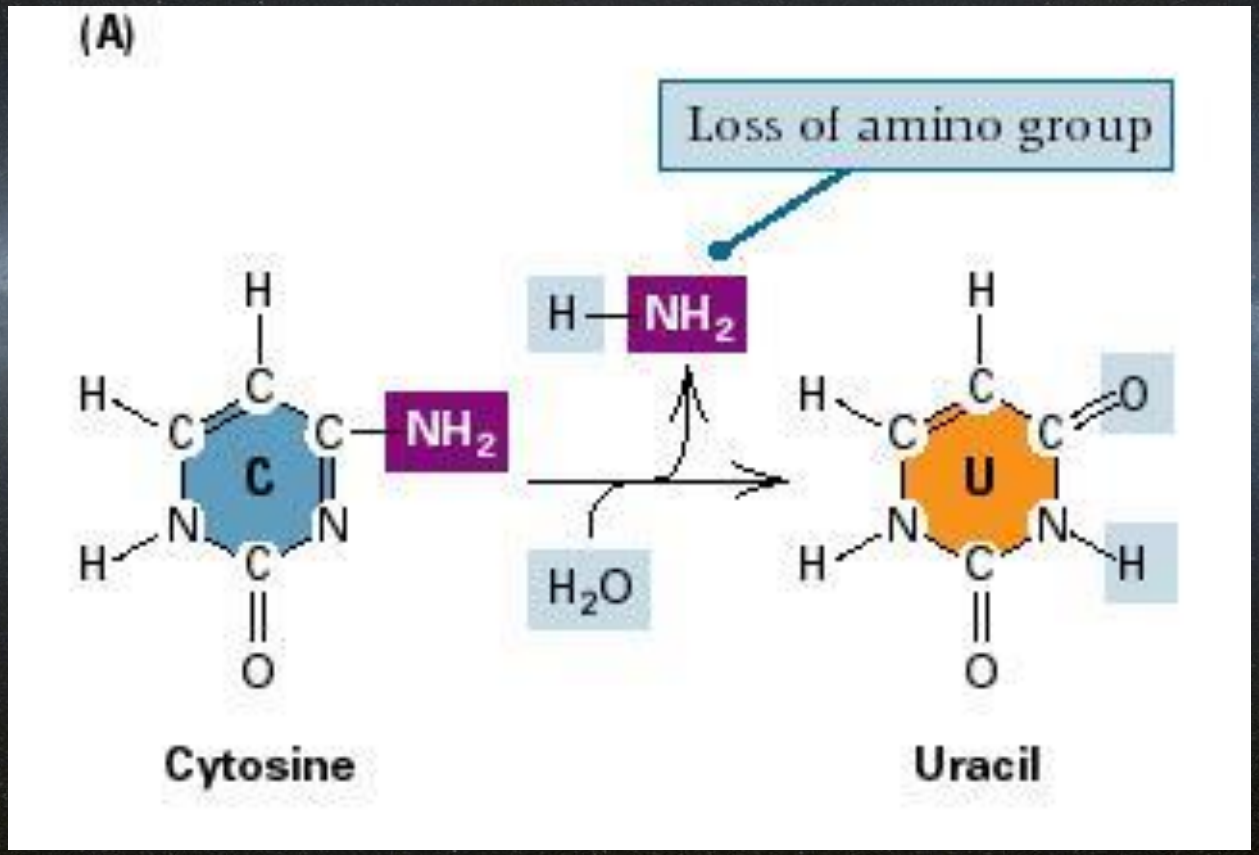
DNA molecule consists of billions of nucleotides, and more than a few breaks a second occur. DNA itself has a measurable half-life - spontaneous depurination can generate abasic sites in DNA strands at an estimated rate of 2,000-10,000 lesions per human cell per day (*Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature 362: 709-715*). Cell is complicated and has an unstable structure. Cell is aging if not frozen, and there are no known exceptions.

DNA breaks

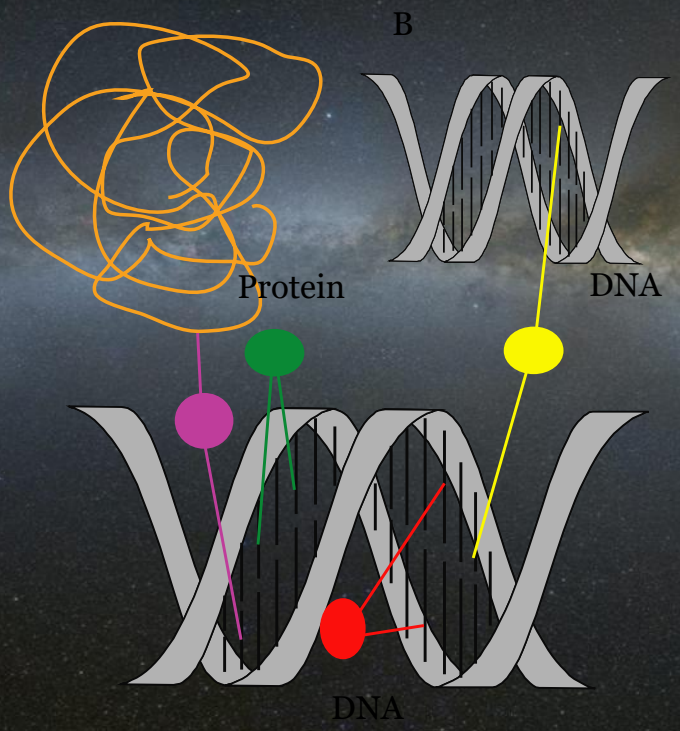

A
Deamination of Cytosine
Clevage of the phosphor backbone
Clevage by depurination and ß-elimination

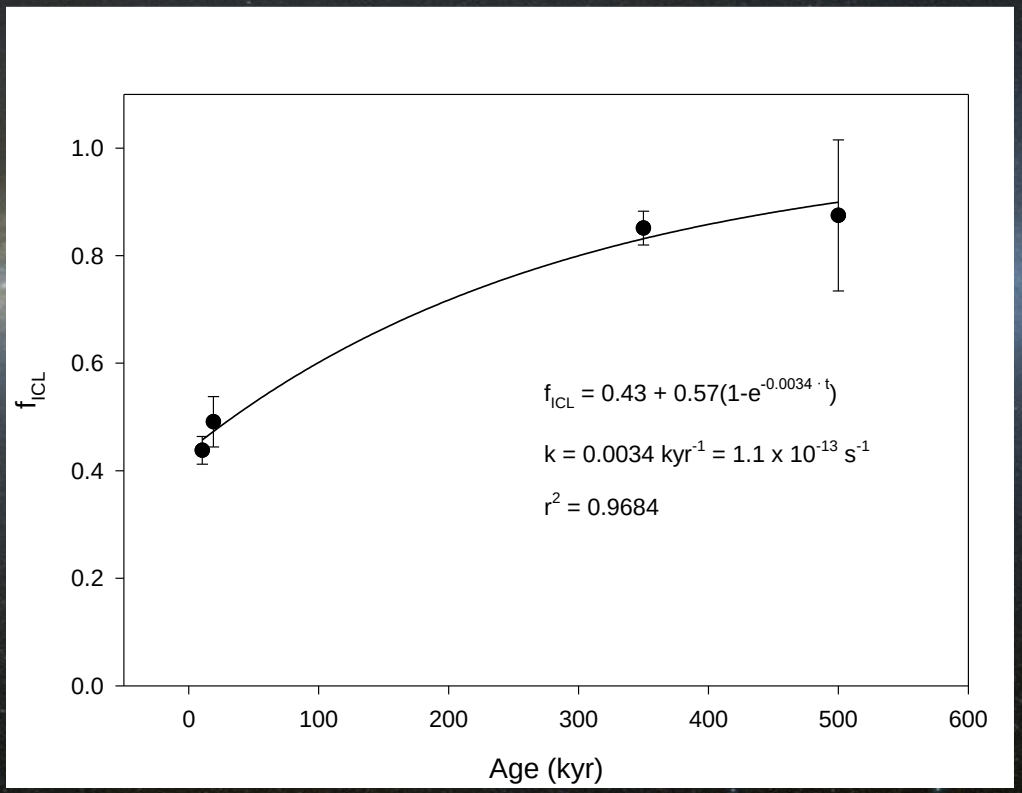
Example: cytosine deamination

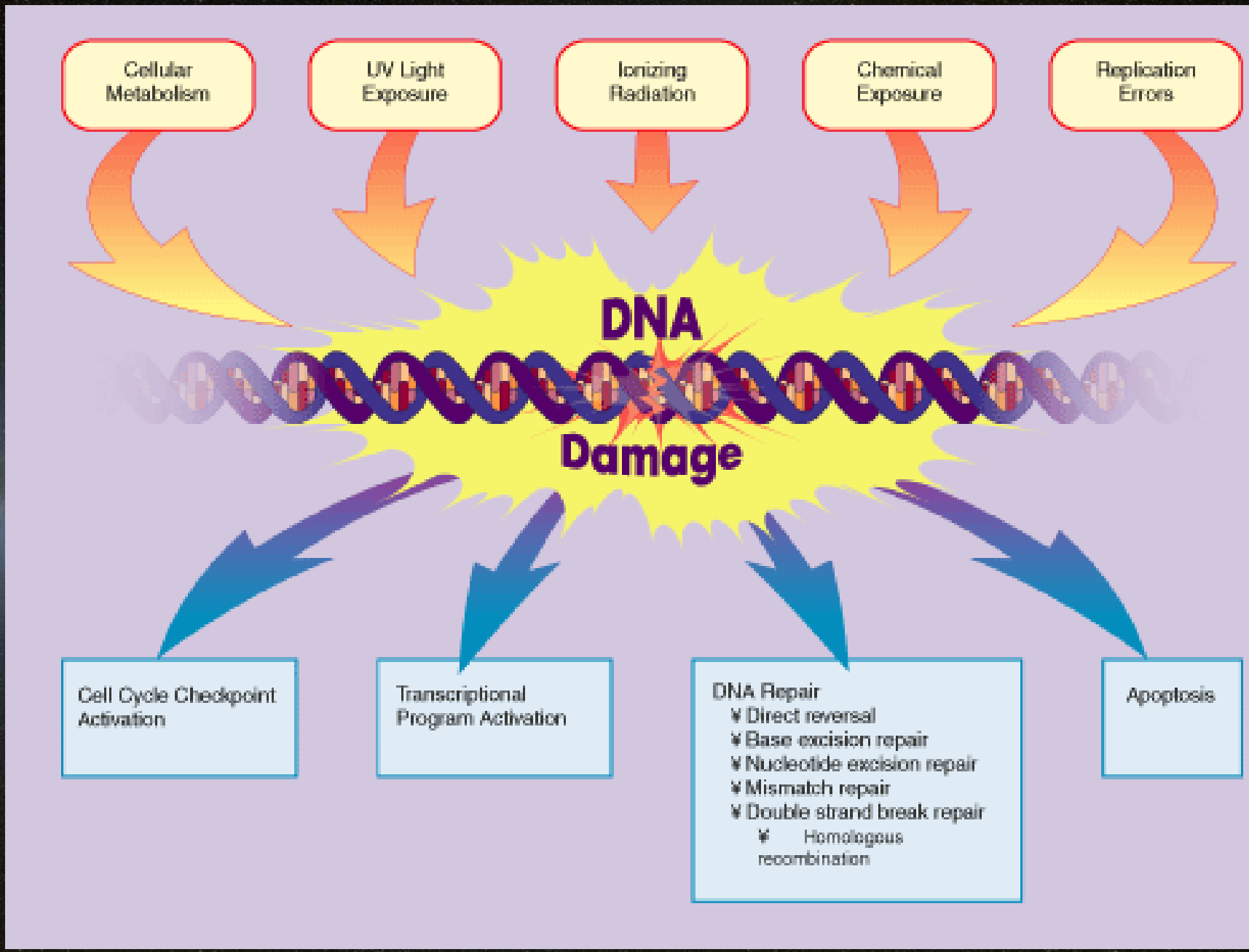
Spontaneous deamination is the hydrolysis reaction of cytosine into uracil, releasing ammonia in the process.



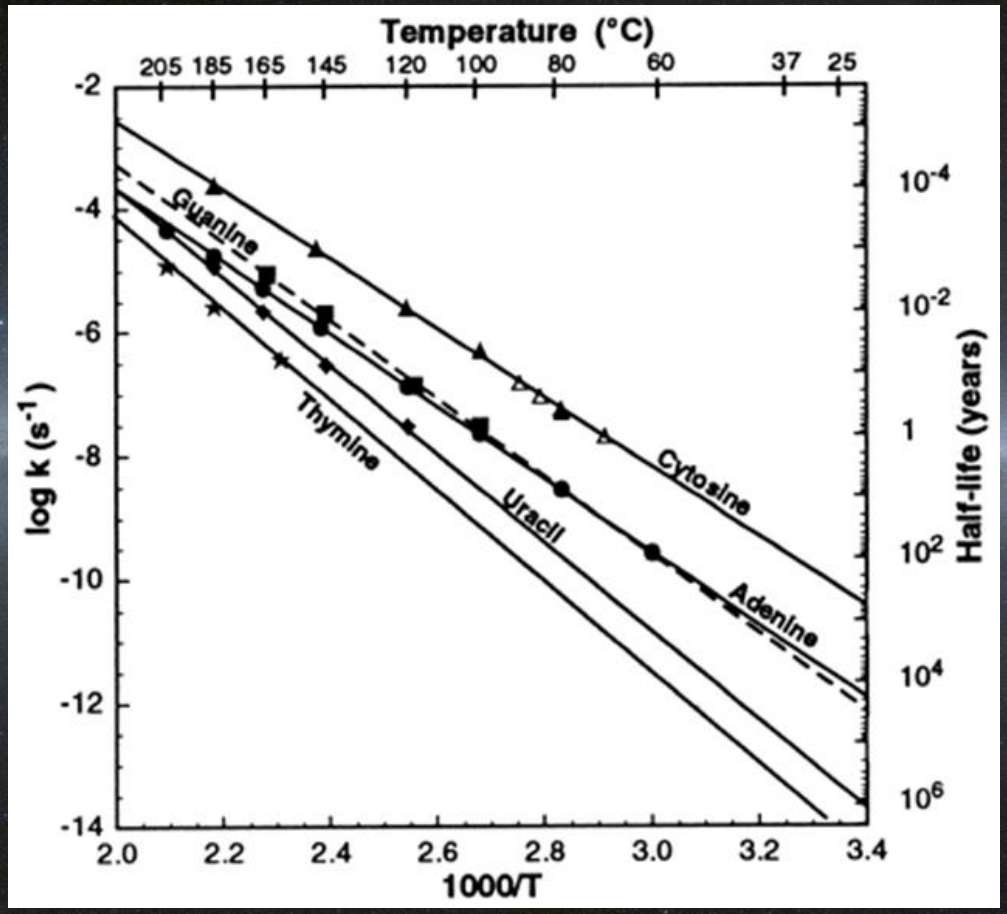
Interstrand Crosslinks
(Denaturation experiment)






Cellular Metabolism
UV Light Exposure
Ionizing Radiation
Chemical Exposure
Replication Errors
DNA Damage
Cell Cycle Checkpoint Activation
Transcriptional Program Activation
DNA Repair
¥ Direct reversal
¥ Base excision repair
¥ Nucleotide excision repair
¥ Mismatch repair
¥ Double strand break repair
¥ Homologous recombination
Apoptosis

DNA nucleotides (A, U, G, C) have a limited life before decomposition as a result of thermal fluctuations (Levy & Miller, 1998)



Most unstable nucleotide is cytosine - it has a half-life of about 500 years at temperatures 0 - -10 degrees C.

Oxidative Decay of DNA

Estimates of the ratio of oxo8dG to dG (for example, in rat tissues) have ranged from approximately 0.25 × 10E5 to higher than 10E4, equivalent to approximately 7,500 oxo8dG or about 1.5 × 10E5 oxidative adducts per human cell, and 150,000 oxidative adducts per cell represents a huge load of damage.

An age-related, persistent 50-100% increase in the steady-state level of adducts is physiologically relevant, representing an inability to prevent or repair oxidative damage. Unfortunately, the detection of such a change in the steady-state frequency of adducts requires the virtual absence of artifactual background noise. Two recent and independent studies, in which the frequency of oxo8dG in a variety of organs of Fisher 344 rats was studied, illustrate this point. In the first, a clear increase in the ratio of oxo8dG/dG was noted (18).

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Constant genomic insults

- At 37°C (normal human body temp) 18,000 purine residues are lost everyday by hydrolysis of the bond connecting the base and the phosphate backbone of DNA
- Transformation of cytosine to uracil by deamination (100-500 times per day per mammalian cell)
- Oxygen free radicals (by products of various metabolic reactions) react with DNA and alter the coding information
- SAM (S-adenosylmethionine) methylates adenine residues some 1200 times per human cell per day.
- DNA replication results in mis-incorporation of bases which if uncorrected would be devastating.
- UV rays can fuse adjacent pyrimidine bases (C,T) generating toxic and mutant lesions.
- IR from earth and cosmic rays can shatter the DNA backbone to form strand breaks or alter the nitrogenous bases
- Occupational exposure to man made chemicals can alter DNA structure

Molecular stability

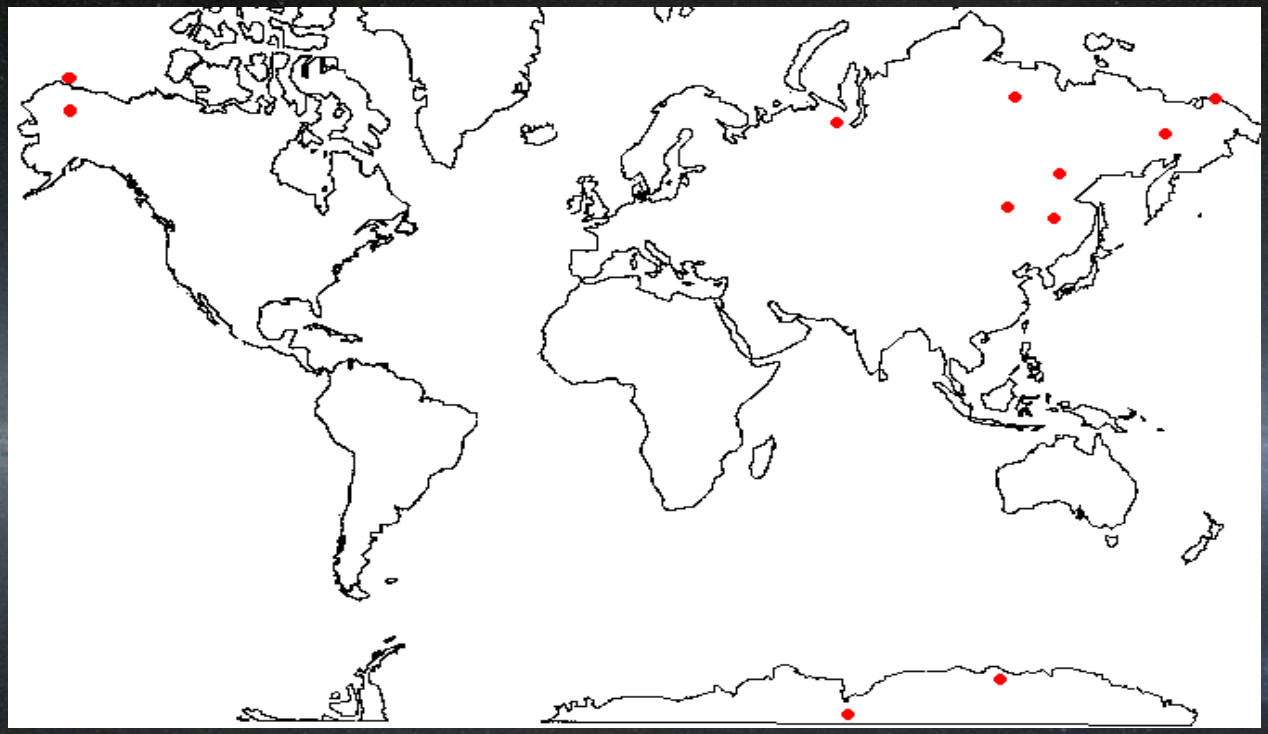
$$k = e^{-\frac{G}{RT}}$$

G – activation energy, kkal/mol;
T - temperature, ° K;
R – gas constant, ~0,001989, kkal/mol*° K

Maximum G is about 45 kkal/mol, but normally it is less; if G=30 kkal/mol, then k is about 300 years

Could microorganisms in permafrost hold the secret of immortality? What does it mean? *Brouchkov A. and Williams P. Contaminants in Freezing Ground. Collected Proceedings of 2nd International Conference*. ***Cambridge, England, 2002***. *Part 1. pp. 49-56*

Viable ancient microorganisms in the world



Viable microorganisms were found in different places (red dots on the map, Gilichinsky and Wagener, 1994) in the world. Oldest ones (up to 3 000 000 years old) have been reported to be found in Kolyma region. Amount of viable cells is up to 10^8 cells in 1 g. They spreads up to 300 m deep in Alaska and exist at temperature –18 –27°C in Antarctic

Diversity of permafrost microorganisms

Types	Locations [ref.]
23 genera, mostly similar to spore-forming *Bacilli or Actinobacteria*	Glacial ice from various locations[16]
Deinococcus, Thermus, Alcaligenes, Cytophaga, Bacteriodes (all psychrophiles)	South Pole snow[11]
Serratia, Enterobacter, Klebsiella, Yersinia (all psychrotrophs)	Ellesmere Island ice[21]
Viable fungi (*Penicillium, Cladosporium, Ulocladium, Pleurotus,…*)	Greenland ice cores; age ≤ 140,000 yr [34]
>57 taxa of eukaryotes (fungi, plants, algae, and protists)	Hans Tausen ice core, northern Greenland[100]
Bacillus and other soil bacteria	At base of Guliya (Tibet) ice core in 1 My-old ice (J. Reeve, personal comm.)
Yeasts, fungi, microalgae, bacteria (including vegetative cells of spore-formers); below 1500 m, only spore- forming bacteria	Vostok ice core[1,2]
Non-spore formers (*Pseudomonas…*); spore-formers (mesophiles to psychrophiles); actinomycetes (psychrotolerant)	Vostok ice core[1]
Caolobacter, an aquatic oligotroph, probably indigenous to Lake Vostok	Accretion ice at bottom of Vostok core (R. Sambrotto, personal comm.)
Aerobic bacteria, mostly psychrotolerant oligotrophic non-sporeformers	Kolyma permafrost[94]
14 diverse genera, dominantly corynebacteria, psychrotrophs, not true psychrophiles †	Kolyma lowland permafrost[87]
11 groups of bacteria including *Proteobacteria and Fibrobacter*; SSU rDNA clones suggest novel genera or families	Kolyma lowland permafrost[104]
>30 genera of great diversity, aerobic and anaerobic, including archaea	Kolyma lowland permafrost[34,95]
Bacillus, Arthrobacter, Streptomyces, inter alia	Antarctic permafrost[95]
Methanococcoides burtonii, Methanogenium frigidum, Halorubrum lacusprofundii	Psychrophilic archaea in Antarctic lakes[28-30]

† Shi et al. (87) concluded that the majority of true psychrophiles are found in the ocean. They are rare in Antarctic rocks and soils and permafrost.

Life in Solid Ice on Earth and Other Planetary Bodies
P. Buford Price
Physics Department, University of California, Berkeley, CA 94720

Decay of DNA

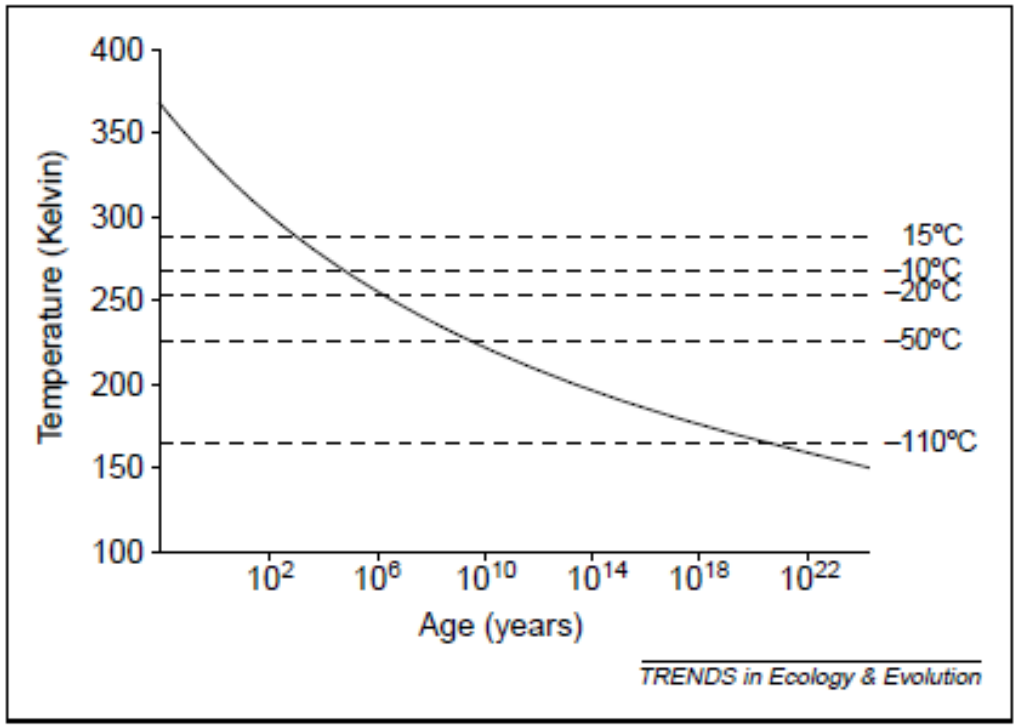


Figure 2. Long-term survival of 100 bp of DNA as a function of temperature. The calculations are based upon a genome size of 3.0×10^6 bp, the Arrhenius equation and depurination kinetics of Lindahl and Nyberg [39] (i.e. a depurination rate of 4×10^{-9} sites sec^{-1} at 70 °C, pH 7.4, and a constant activation energy of 31 kcal mol^{-1}). We have simplified calculations assuming damage is distributed equally over the genome at all purine sites.

Ancient DNA survival

Plants (*rbcL* about 130 bp):

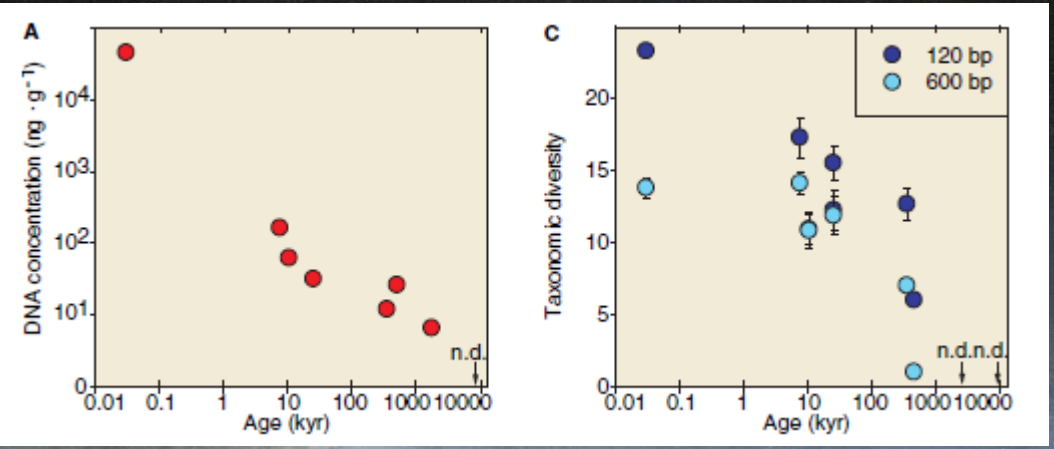
- PCR products up to 300-400 kyr (including NZ cave site)
- No PCR products million year old samples

Animal (mtDNA 88-234 bp):

- PCR products up to 20-30 kyr
- no PCR products 300-400 kyr and million year old samples

Bacterial DNA concentration and time relationship in permafrost

The persistence of bacterial DNA over geological timespans remains a contentious issue. We present the study of DNA durability and degradation of a broad variety of bacteria preserved under frozen conditions, using ancient DNA methods .



Current Biology Vol 14 No 1

Long-term persistence of bacterial DNA Eske Willerslev1,2, Anders J. Hansen1*, Regin Rønn1, Tina B. Brand1, Ian Barnes2, and others

Bacterial DNA survival only

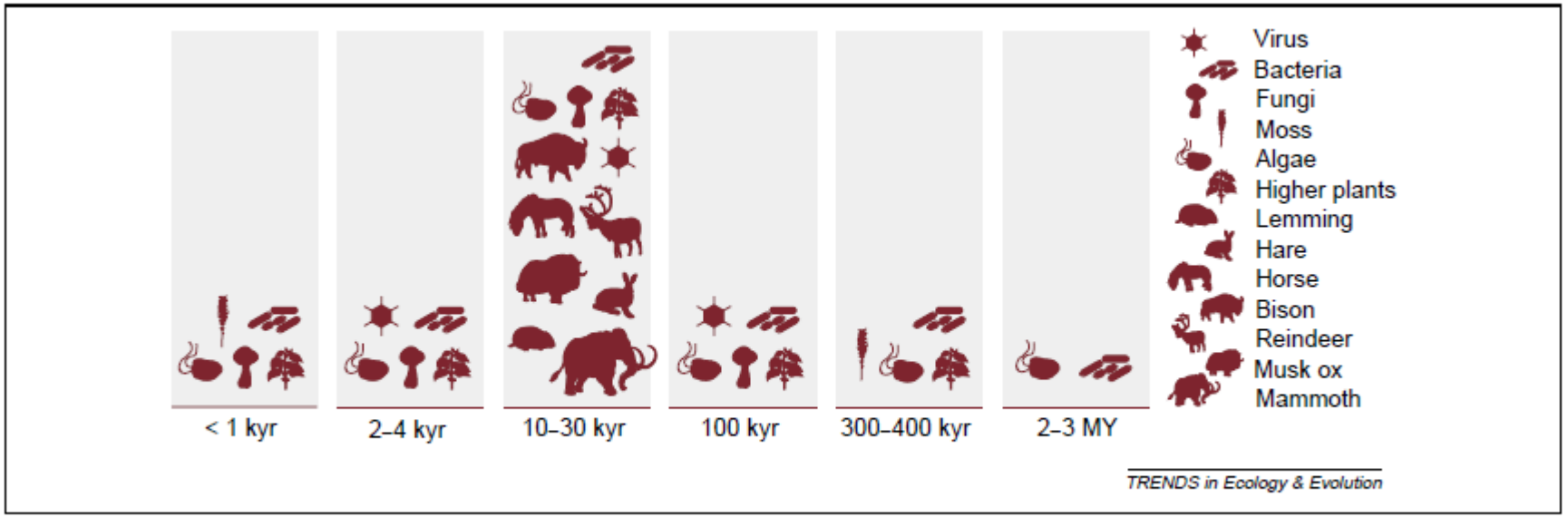


Figure 1. DNA and/or RNA sequences and viable cultures reported from glacial ice and permafrost of various ages [1–15]. This does not include the many DNA sequences from bone and soft tissue remains from permafrost settings. Abbreviations: Kyr thousand years BP; MY millions of years.

References

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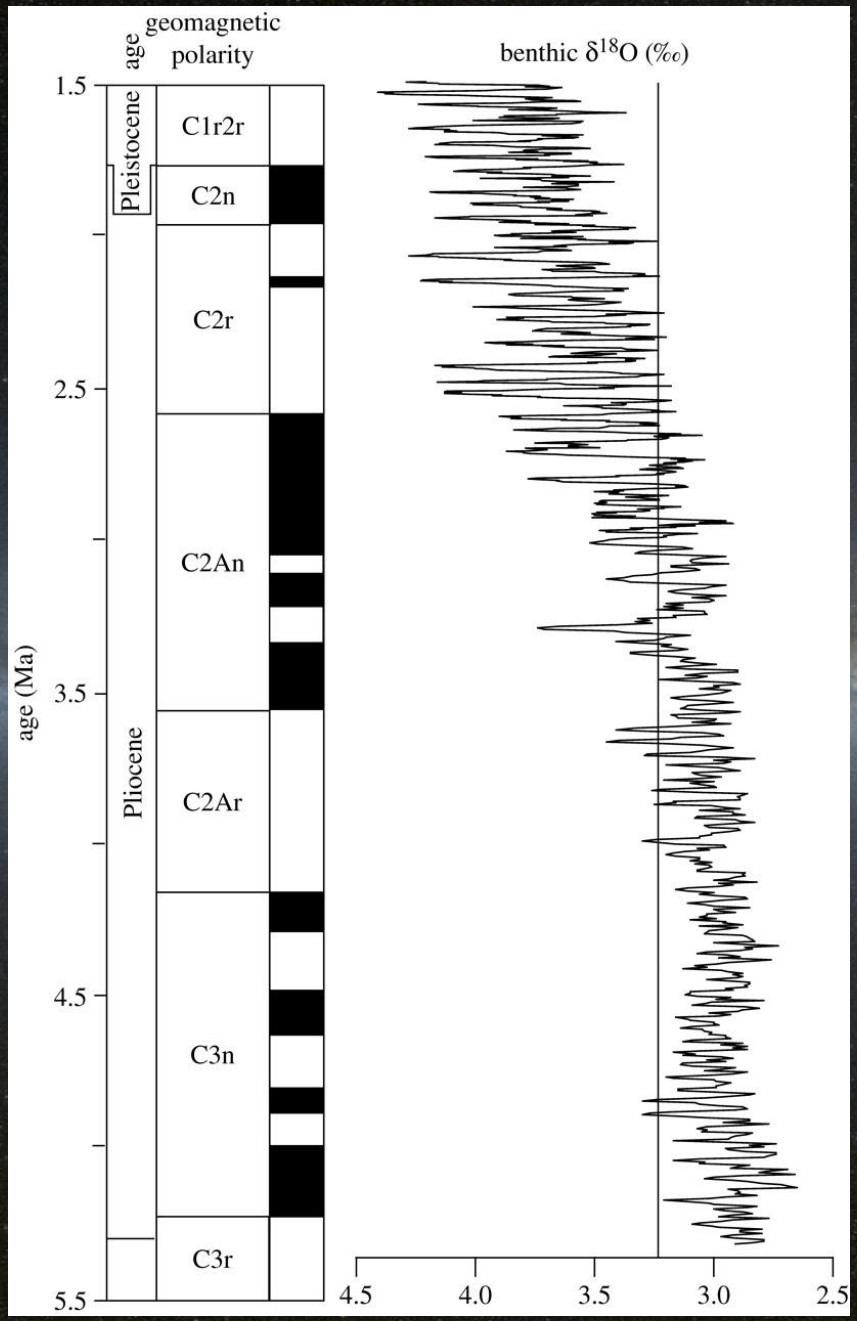
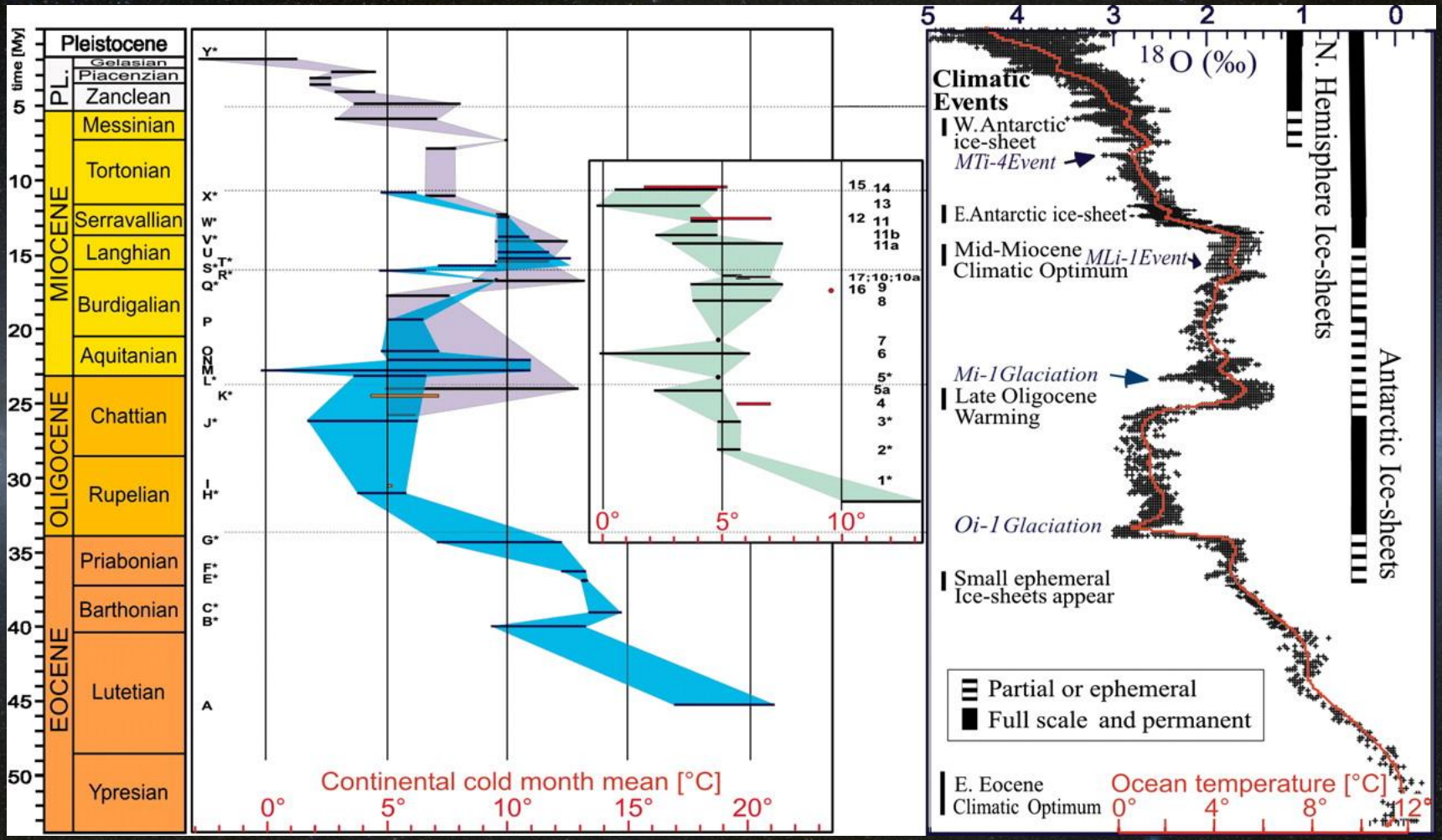
2 Abyzov, S.S. (1993) Microorganisms in the Antarctic ice. In *Antarctic Microbiology* (Friedmann, E.I., ed.), pp. 265–295, Wiley-Liss
3 Shi, T. *et al.* (1997) Characterization of viable bacteria in Siberian permafrost by 16S rDNA sequencing. *Microb. Ecol.* 33, 169–179
4 Vorobyova, E. *et al.* (1997) The deep cold biosphere: facts and hypotheses. *FEMS Microbiol. Rev.* 20, 277–290
5 Castello, J.D. *et al.* (1999) Detection of tomato mosaic virus RNA in ancient glacier ice. *Polar Biol.* 22, 207–212
6 Ma, L.J. *et al.* (1999) Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* 92, 286–295

Search for the oldest permafrost

Mammoth Mountain in Eastern Siberia; neogene alluvial deposits aged about 10 millions years



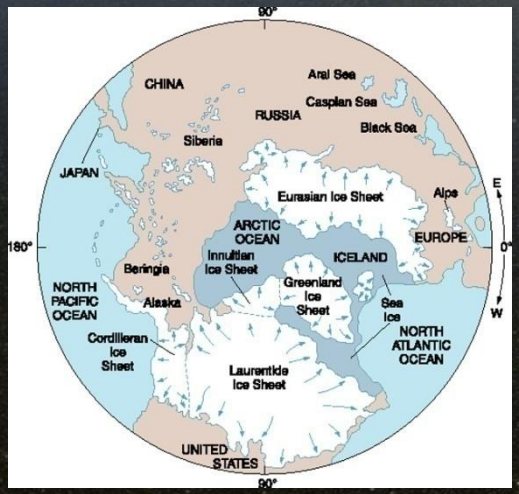
Pliocene magnetochronologic framework, after Berggren et al. (1995). Benthic δ18O record from Lisiecki & Raymo (2005). Vertical line through isotope curve represents present-day value.

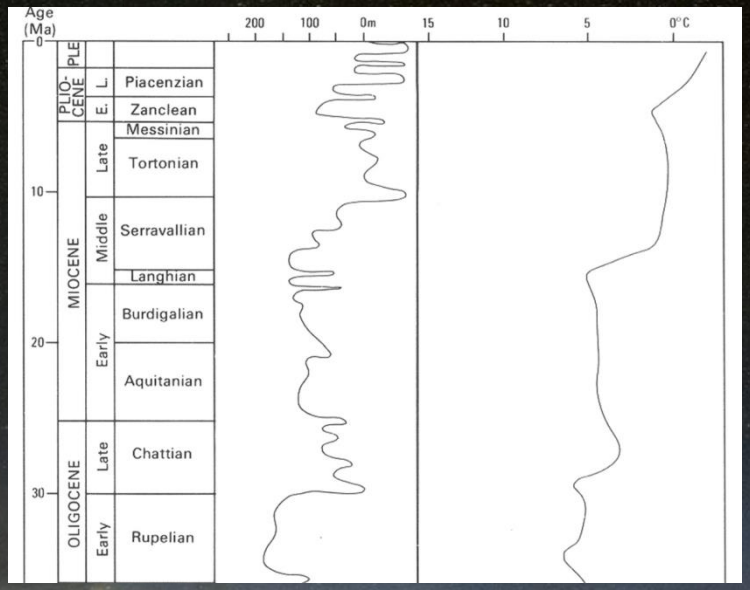


Cooling in Neogene and Pleistocene

All Pleistocene warm time intervals are characterized by the co-occurrence of temperatures of 3°C above preindustrial levels (P.P.Smolka, Cold aspects of Neogene and Pleistocene warm climates IGC 2008)

Cold climate of Pleistocene was established 2-3 million years ago (O.Ivashenko, Climate warming)





Eustatic sea-level curve (left) and ocean bottom water temperature (right) last 30 millions years (after Haq et al. 1987; Savin 1977).

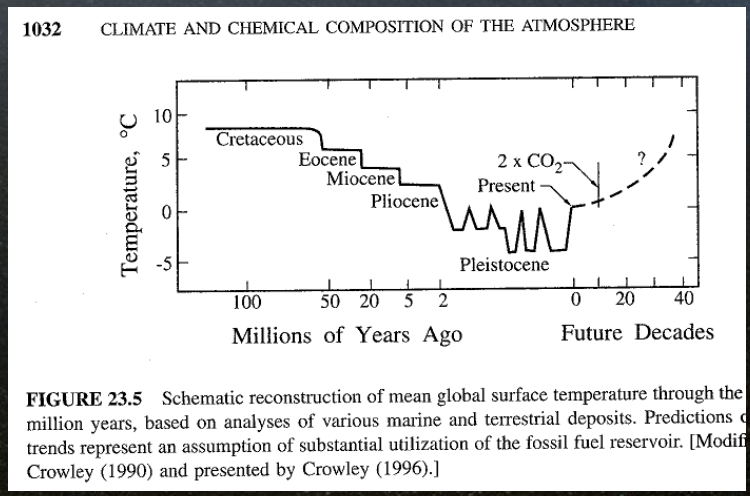
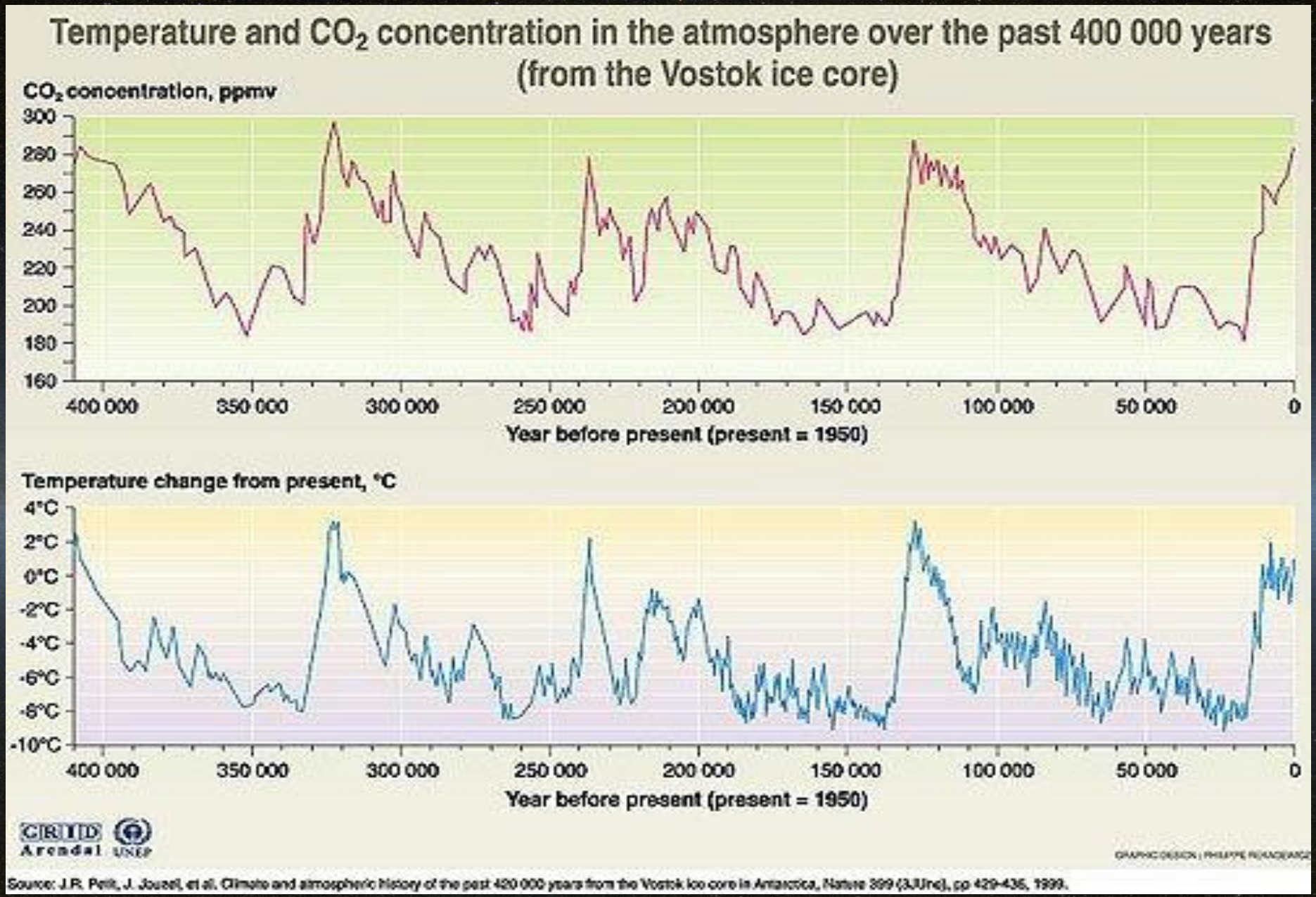
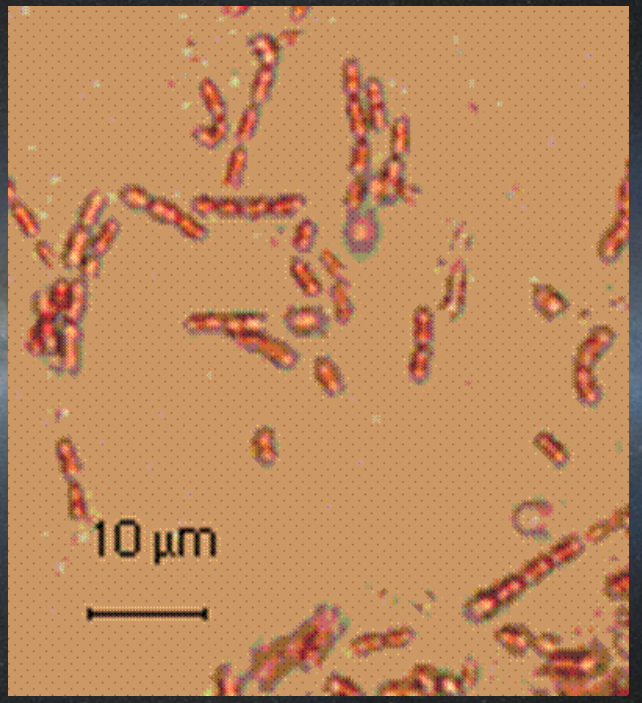


FIGURE 23.5 Schematic reconstruction of mean global surface temperature through the million years, based on analyses of various marine and terrestrial deposits. Predictions trends represent an assumption of substantial utilization of the fossil fuel reservoir. [Modif Crowley (1990) and presented by Crowley (1996).]



J. R. Petit, et al, "Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica" in Nature 399, pg 429 (3 June 1999).

A bacterium found in the ancient permafrost



Isolated strain of *Bacillus sp.*;
right – Gram staining; left – growth in Petri dish

16S rRNA homology to known species

Name	Length	Identities	Homology %
Bacillus sp. 19489 16S rRNA gene	1503	1441/1471	97
Bacillus sp. LMG 21002, partial 16S rRNA gene	1503	1439/1471	97
Bacillus sp. 19491 16S rRNA gene	1503	1438/1471	97
Glacial ice bacterium SB150-2A2	1459	1438/1471	97
Bacillus sp. 19494 16S rRNA gene	1503	1437/1471	97
Bacillus sp. B90 16S rRNA gene	1462	1436/1470	97
Bacillus simplex 16S rRNA gene, strain DSM 1321T	1522	1415/1444	97
Bacillus sp. PAMU-1.13 gene	1504	1436/1471	97
Bacillus macroides strain JPL-4 16S rRNA gene	1475	1435/1471	97
Uncultured soil bacterium clone 1296-1	1474	1429/1464	97
Bacillus sp. 'kanghwensis' 16S rRNA gene	1564	1434/1471	97
Bacillus macroides rRNA gene	1443	1425/1459	97
B.macroides (NCDO 1661)	1478	1422/1459	97
Glacial ice bacterium G500K-17	1436	1402/1433	97
Glacial ice bacterium SB100-8-1-1	1462	1426/1473	96

DNA conservation? Adsorbed on clay minerals?

Published online May 6, 2005

Binding of DNA from *Bacillus subtilis* on Montmorillonite–Humic Acids–Aluminum or Iron Hydroxypolymers: Effects on Transformation and Protection against DNase

Carmine Crecchio,* Pacifico Ruggiero, Maddalena Curci, Claudio Colombo, Giuseppe Palumbo, and Guenther Stotzky

ABSTRACT

The equilibrium adsorption and binding of DNA from *Bacillus subtilis* on complexes of montmorillonite–humic acids Al or Fe hydroxypolymers (Al–M–HA or Fe–M–HA) at different M/HA ratios, the desorption of DNA, the capacity of bound DNA to transform competent cells of *B. subtilis* in vitro, and the protection of bound DNA from degradation by free and organomineral-bound DNase I are reported. Adsorption was rapid (maximal after 2 h), occurred from pH 3 to 10, and was higher on Al–M–HA than on Fe–M–HA. Saturation of the sites on the surface or between the layers of Al– or Fe–M–HA occurred with only some complexes, depending on how the complexes were prepared. Essentially no desorption under stringent conditions was observed. Bound DNA transformed auxotrophic competent cells of *B. subtilis*, although at a lower frequency than free DNA. Bound DNA was protected more than free DNA against degradation by DNase I, and differences in resistance to degradation between free and bound DNA were more evident when DNase was also bound on the organomineral complexes.

Despite the relatively large number of papers dealing with the adsorption of DNA on clays and HA, essentially no information is available about the adsorption of DNA on organomineral particles, probably the dominant form of clays and HA in soil. The influence of Al and Fe in the intercalation of HA in the swelling clay and in the sorptive properties is still not fully understood, despite many studies of sorption/desorption of organic compound by synthetic HA-clay model sorbents (Mortland, 1970; Murphy et al., 1990; Goldberg et al., 1999).

The release of DNA from plants, animals, and microorganisms can occur by lysis after their death, after infection of bacteria by phages (Redfield, 1988), and by active release of plasmid and chromosomal DNA by living bacteria (Lorenz et al., 1991). Such extracellular DNA can attain concentrations that could result in horizontal gene transfer (HGT) by transformation. Numerous bac-

Permafrost ice – arena for biological selection?

Billions of bacterial cells in permafrost have been trapped in ice for thousands of years. Only those which survived have a mechanism of repair.

DNA repair?

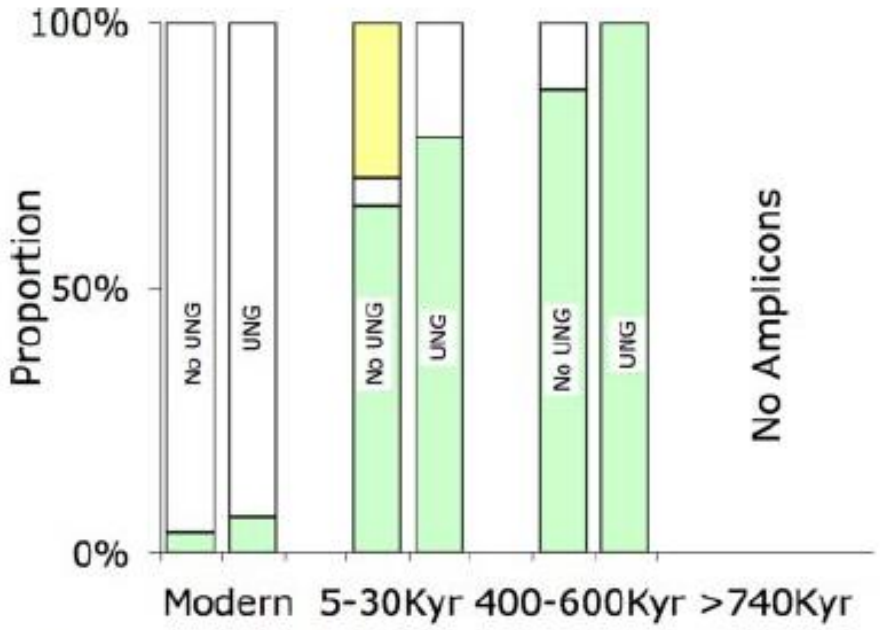


Fig. 3. Proportion of clones before and after UNG treatment (see Fig. 2). Low-GC Gram-positive bacteria (yellow) such as the endospore-former *Clostridia* exhibited DNA damage. Gram-negative bacteria (white) and high-GC Gram-positive bacteria (green) such as *Actinobacteria* have no known capacity for dormancy.

Johnson *et al.*

Ancient bacteria show evidence of DNA repair

Sarah Stewart Johnson*†, Martin B. Hebsgaard†, Torben Christensen‡, Mikhail Mastepanov‡, Rasmus Nielsen†, Kasper Munch†, Tina Brand†, M. Thomas P. Gilbert†, Maria T. Zuber*, Michael Bunce§, Regin Rønn†, David Gilichinsky¶, Duane Froese||, and Eske Willerslev†**

Practical use?

J Microbiol Immunol Infect
2005;38:96-104

Antitumor features of *Bacillus oligonitrophilus* KU-1 strain

Sergey V. Malkov[1], Vladimir V. Markelov[2], Gleb Y. Polozov[1], Larisa I. Sobchuk[1], Natalia G. Zakharova[1], Boris I. Barabanschikov[1], Alexander Y. Kozhevnikov[3], Rauf A. Vaphin[1], Maxim V. Trushin[1,3]

[1]Department of Genetics, Kazan State University, Kazan; [2]Kazan Municipal Rehabilitation Medical Health Center "Sanatorium Krutushka", Kazan; and [3]Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

Received: August 20, 2004 Revised: November 10, 2004 Accepted: November 26, 2004

Unexpected approach…

By: Provenance
Pack: 60-ml
Product Code: PVN2320
Price: £19.99

Description

Provenance Objectives Anti-Aging Cream is a highly advanced formula that will truly make your skin smoother and more youthful looking - IN ONLY 30 DAYS!

Key Ingredients

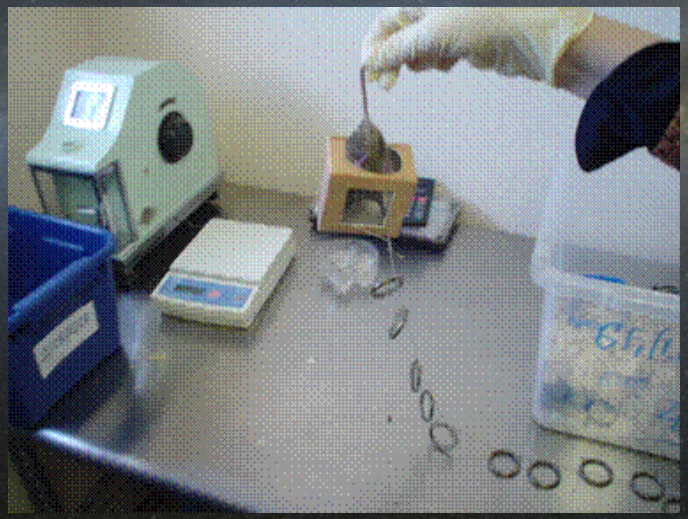
Aqua, C12-15 Alkyl Benzoate, Niacinamide, Glycerin, Cyclomethicone, Cetearyl Alcohol, Cetyl Alcohol, Ceteareth-20, DEA Cetyl Phosphate, Tocopheryl Acetate, Stearic Acid, Borago Officinalis, Linum Usitatissimum, Aloe Barbadensis, Retinyl Palmitate, Tocopherol, Dimethicone, Nordihydroquaiaretic Acid, Oleanolic Acid, Bacillus Ferment, Caprylyl Glycol, PEG-60 Almond Glycerides, Propylene Glycol, Butylene Glycol, Phenoxyethanol, Carbomer, Parfum, Methylparaben, Propylparaben, Disodium EDTA.

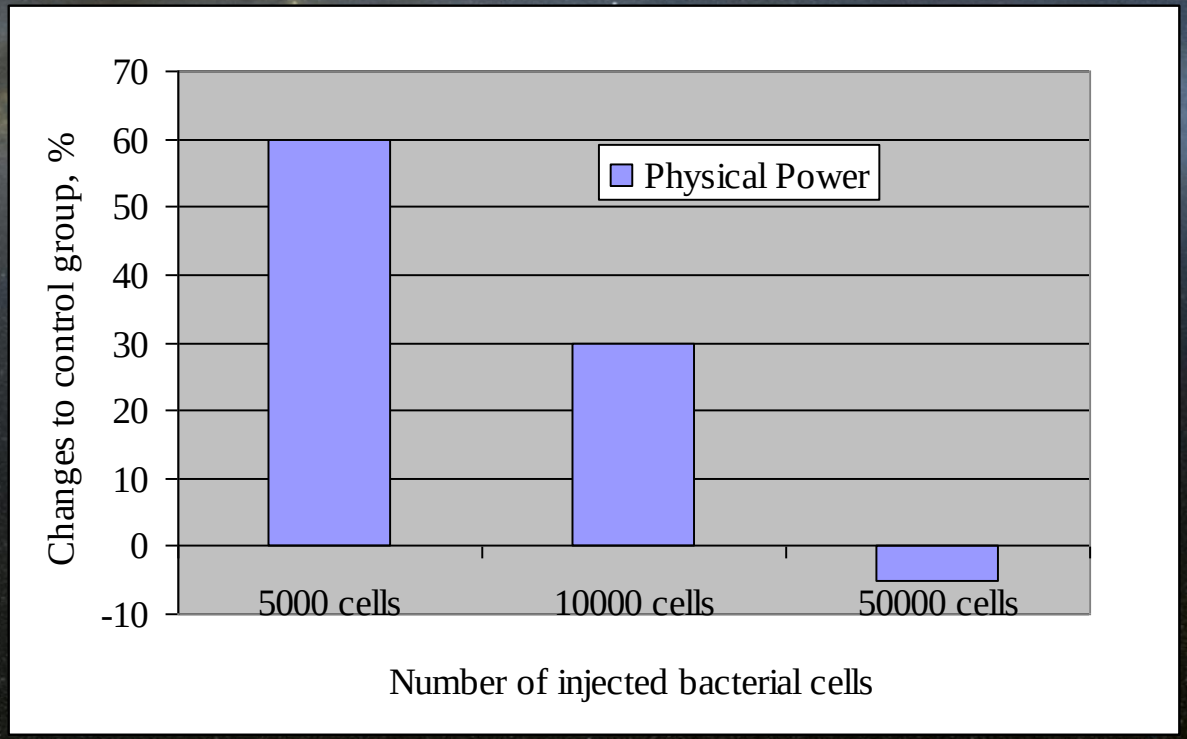
Some preliminary results:

- Immunostimulation
- Anti-tumor effect
- Impact on longevity
- Increased physical activity
- Wound recovery
- …

Increase in physical strength in mice

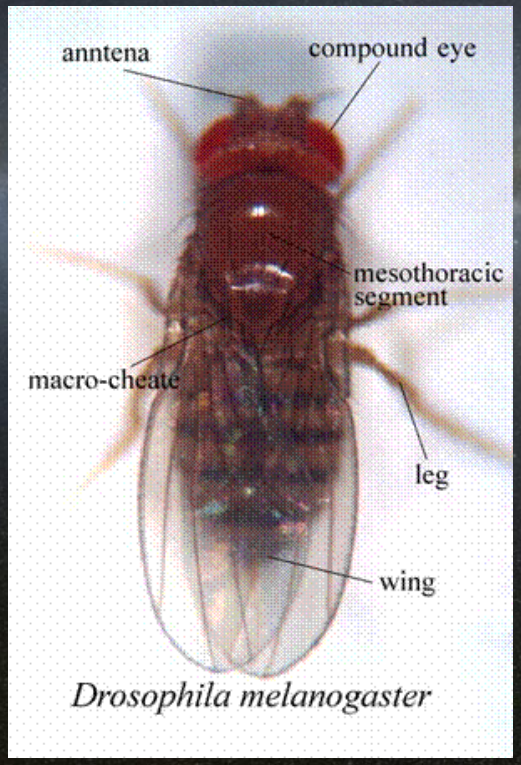
Physical activity after one injection (3 groups of mice): Biggest response on the smallest amount of bacterial cells.

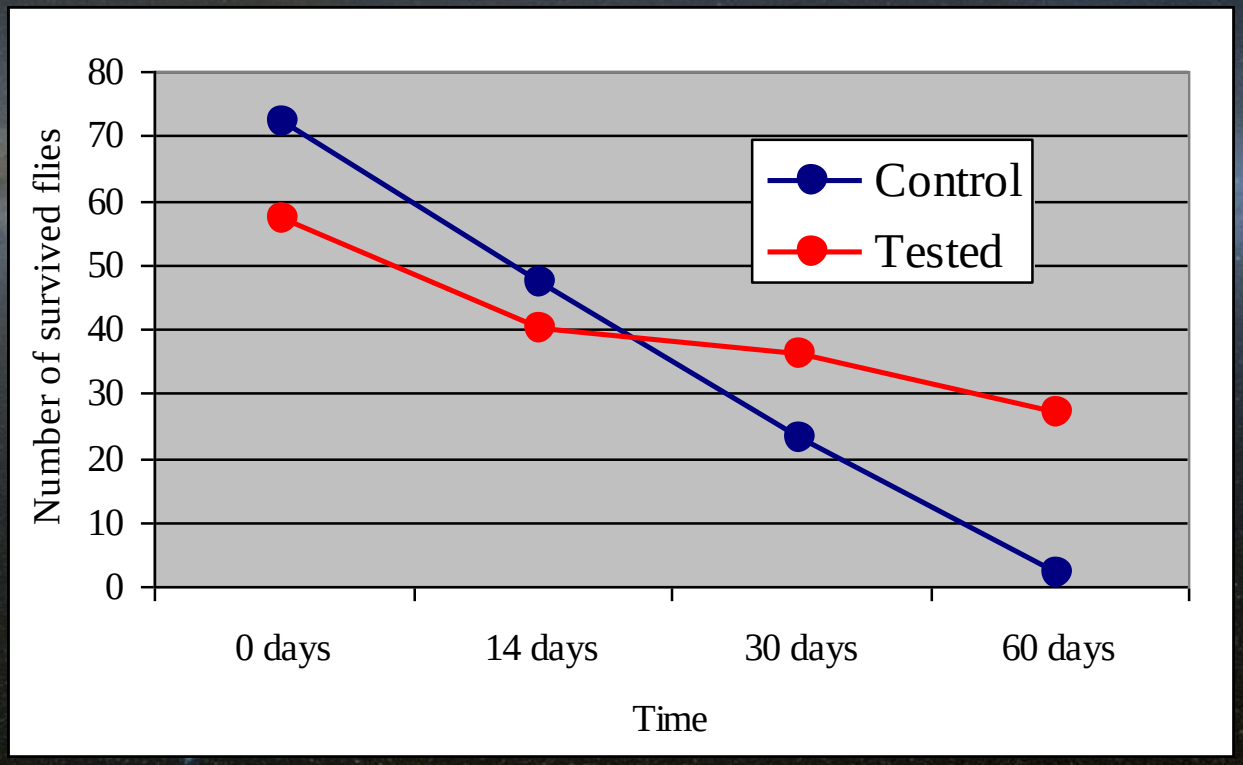




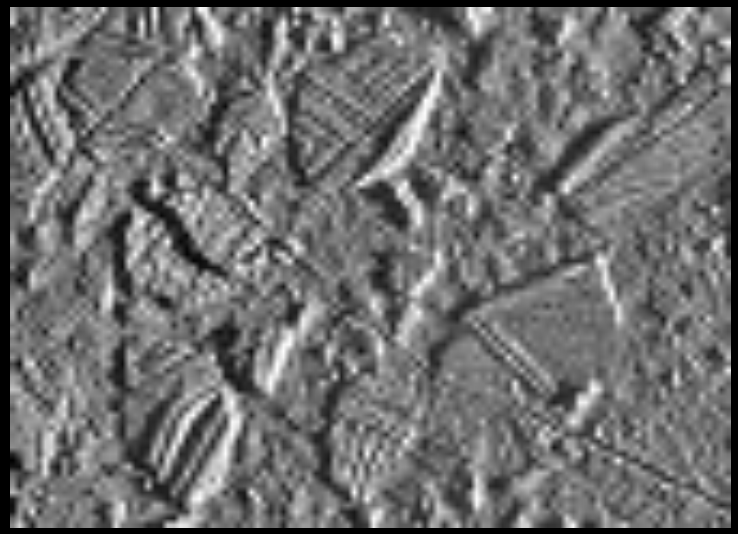
Increased longevity of Drosophila

- up to 6 months, 2 months is normal

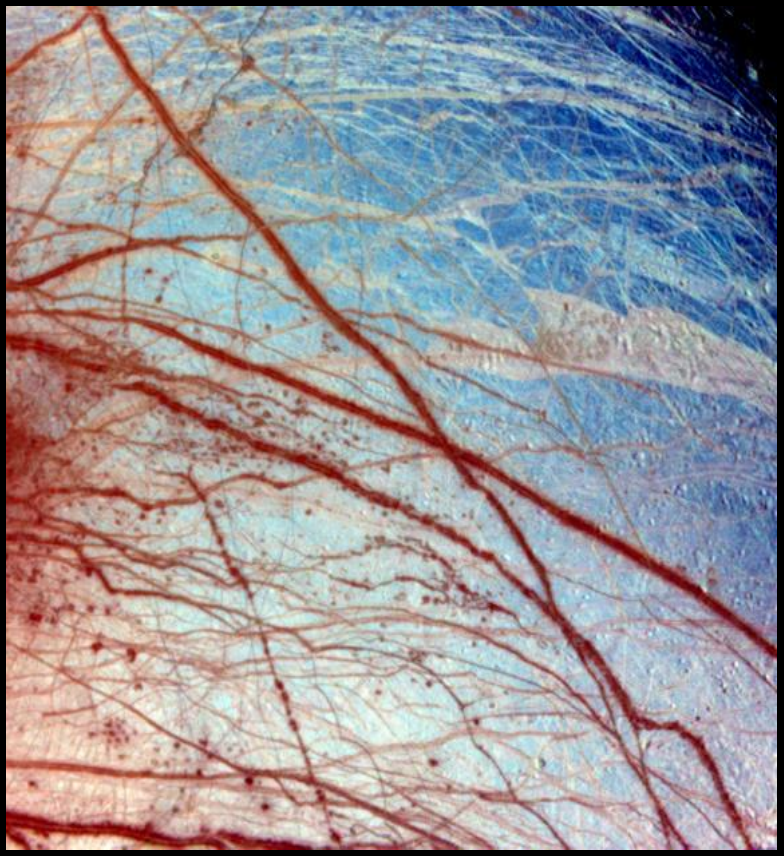




Chaotic terrain on Europa

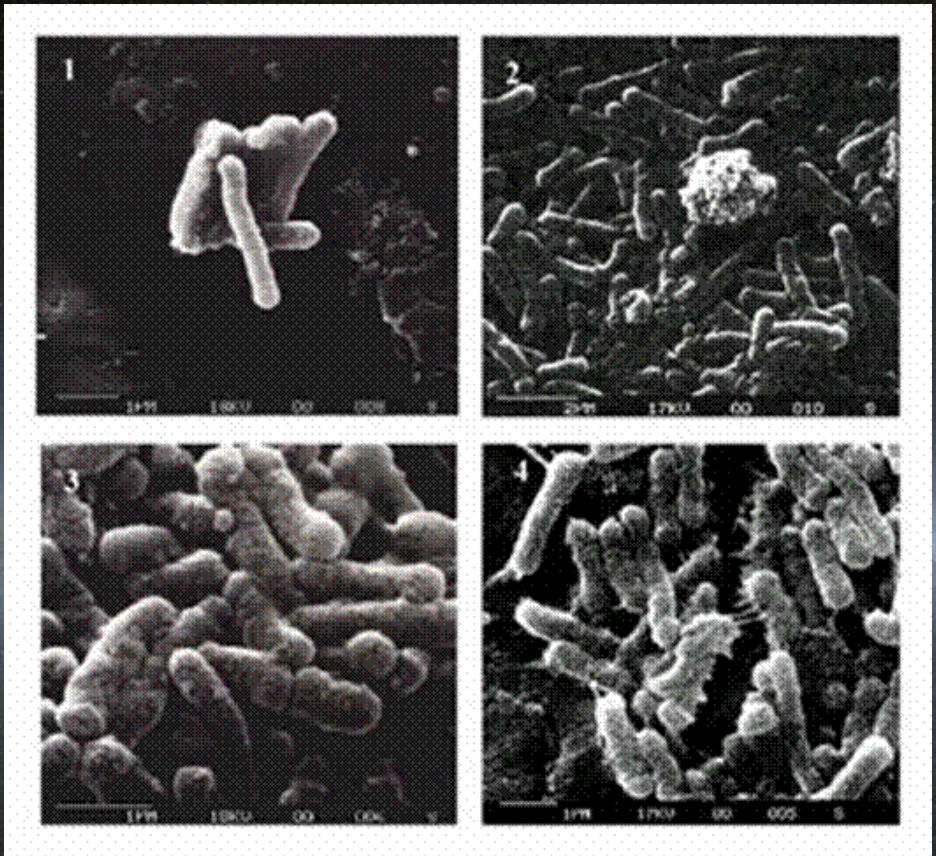


Courtesy: James Granahan



Comets: coming from the Cold

Bacteria-like structures have been found in meteorites



(MetA. Iperstenic chondrite, which fell on Feb. 3, 1882 at Mocs, Transilvania and MetC. Enstatitic olivinic chondrite, which fell in 1919 at Bur Hacaba, Somalia (kindly provided by Real Museo Mineralogico, Naples)


Thank you!