Subsurface martian soil as favorite place of terrestrial radioresistant bacteria origin.

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WHAT FEATURES OF TERRESTRIAL MICROORGANISMS COULD BE AN EVIDENCE OF THEIR EXTRATERRESTRIAL ORIGIN?

*One possible clue is the ability to adapt to environments that could never have happened on Earth.*

High radioresistance – tolerance to ionizing radiation (p.n, $\gamma$-rays)

- Ionizing radiation is specific stress in comparing to other stresses because of the energetic particles are able to break the DNA by direct hits. This type of damage can not be prevented by any biochemical mechanism.
Extremely radioresistant bacteria

- *Deinococcus radiodurans*, *Rubrobacter radiotolerance*, *Rubrobacter xylanophilus*, *Chroococcidiopsis*, *Termococcus gammatolerance*. Radioresistance is 100-1000 times higher than other microorganisms.

- High doses of ionizing radiation create a lot of DNA damages and radioresistant bacteria have an unknown and unique mechanism for DNA repair (more 100 double strand breaks).

- Lethal radiation dose >> doses accumulated during the lifetime of the radioresistant bacteria (by 10 orders of magnitude). Time of accumulation of the lethal doses is $10^6$ – $10^8$ years.

- High radioresistance - totally unnecessary ability on Earth.
Is Radioresistance a “side-effect”?

• Hypothesis: Radioresistance – “incidental” side-effect of adaptation to an extreme desiccation in the unknown natural habitats (Mattimore and Battista, 1995).

• Reasons - general tendency of the radiation-sensitive mutants of the Deinococcus radiodurans to possess a lower resistance to desiccation (comparing to the “wild” type).
DIFFICULTIES

Lack of correlation between survival under extreme desiccation and irradiation. Data points were taken from Mattimore and Battista (1996) and Mattimore et al. (1995)
Moreover, Battista et al. (2001) showed in later experiments that *D. radiodurans* mutants that were more sensitive to desiccation possessed a higher radioresistance ability than the wildtype strains.

These researchers concluded that genes responsible for radioresistance are most likely different from genes responsible for desiccation resistance.

Radioresistance has not been shown to be induced in any nonradioresistant bacteria as a result of desiccation or by cycles of desiccation/hydration.
• Bacteria that survive extreme desiccation on Earth and even desiccation in the vacuum of space (Horneck et al., 2002) do not possess high radioresistance ability.

• *Rubrobacter*, *Thermococcus*, and *D. Radiodurans* do not require desiccation resistance in their modern natural habitats (*e.g.*, hot springs, deep-sea hydrothermal vents, Antarctic soil) or in man-made habitats (*e.g.*, canned meat).

• Different types of radioresistant bacteria have distant phylogenetic relationships and prefer different environments. Thus, they could not have inherited radioresistant ability from each other (Ferreira et al., 1999).

• Hence, hypothetical “incidental phenomena” must have taken place several times during the evolution of life.
Our hypothesis
Pavlov et. al. 2006

• 1. High radioresistance is not a side-effect but a result of the adaptation process to the high radiation background and regular variations of climate and atmospheric pressure on Mars.

• 2. Radioresistant bacteria have originated on Mars and were transferred to Earth by Martian meteorites.
How radioresistance can be trained?

• The radioresistance of the “normal bacteria“ can be increased dramatically using subsequent series of the sublethal irradiation cycles.

• Laboratory cycle: Escherichia coli and two types of Bacillus were irradiated by high doses of $\gamma$-radiation.

• Irradiation was shut down after 99.9 % of the population were dead in each cycle. Survived bacteria were re-grown to their initial population and cycle was repeated.
Surviving fraction (S) of the bacterial population versus accumulated radiation dose.
Is There a Natural Environment Where it Could Have Happened?

• Intensive ionizing radiation background
• Natural cycle: regular long periods of cold and/or dry conditions → dormancy state of bacteria → mechanism of DNA repair do not work → accumulation of sublethal dose of irradiation.
• Short period of favorable conditions → recovery of population
Great oscillations in Martian obliquity (period $1.2 \times 10^5$ years) → oscillations of annual insulation of the polar regions → dramatic regular oscillations of global climate and atmospheric mass → long periods of the “frozen state” for subsurface layers → long periods of the bacterial dormancy

Atmospheric thickness < 1 g cm$^{-2}$ during the cold periods, no magnetic field →

Irradiation of cosmic rays in subsurface layers of Mars 100-fold of the terrestrial irradiation. → Periods of sublethal doses accumulation $\geq 10^4$ years. → Total time of “training process” (100 cycles) $\geq 10^6$ years
Transfer of Martian Biota

- Transfer of microorganisms from Mars to Earth and backward by meter-size meteorites have been considered in Mileikowsky, et al., (2000); Horneck .et al., (2002)

- More than $5 \times 10^7$ non-sterilized debris of the Martian surface rocks were able to arrive on Earth and about $10^5$ terrestrial meteorites were able to arrive on Mars during last 4 Ga.
Protection mechanisms for bacteria during space travel

- Against UV-radiation – a few mm of substance is a good shield
- Against Cosmic rays – about 1m substance is good protection in large-size meteorites.
- Against Vacuum – spore formation, protective molecules, DNA repair. Spores survived in space experiments
Survival launch and landing

• Pressure and acceleration are not limiting factors (experiments of Horneck et al, Roten et al, Setlow)

• High temperature – internal part of several martian meteorites was not heated to more than 100°C

Transfer of biota between Mars and Earth is possible because launch, landing and space hazards are not critical for microorganisms
Why the genome of “Martian bacteria” looks like the genome of terrestrial bacteria?

• Life had originated on Mars. Earth was infected by martian biota. Transfer of the martian microorganisms to Earth took place many times.

• Life had originated on Earth. Early warm and wet Mars was infected by the terrestrial biota. Terrestrial microorganisms evolved on Mars and obtained high radioresistance. Then, they were transported back to Earth.

• Early Earth and Mars were infected from the common external source.
Could be the short periods of “favorite conditions” in subsurface Martian soil during recent history of Mars?

Environmental conditions on the surface of modern Mars

— very low atmospheric pressure 3–7 mbar => liquid water is unstable at the surface. Extremely dry atmosphere.

— high UV flux on surface + superoxidants: $O_2^-$, $H_2O_2$, $NO_x$ => upper dust mixed layer of martian soil is sterilized;
— weak magnetic field, thin atmosphere => 100 times more intensive flux of ionizing radiation than on the Earth’s surface

Any known living forms can’t exist on the Martian surface => shallow subsurface?
Water ice on Mars

Typical ground ice table depths vary from a few cm to 1 m (Boynton et al., 2002; Feldman et al., 2002; Mitrofanov et al., 2002).
— Weak greenhouse effect and lower solar radiation input $\Rightarrow$ global average temperature $\langle T \rangle = 220\, \text{K}$, $T_{\text{min}} \sim 150\, \text{K}$, $T_{\text{max}} \sim 290\, \text{K}$, diurnal variations about $100\, \text{K}$.

Daytime Temperature

Thermal Emission Spectrometer data
Laboratory modeling

The main goal of the experiment was to study low atmospheric pressure effect on the activity of microorganisms in the simulated subsurface martian permafrost.

Our experiment
Pavlov et. al. 2010

- Simulation of Martian subsurface conditions in the vacuum chamber at atmospheric pressure down to 0.01-0.1 mbar

Low atmospheric pressure
Wide range of temperature variations
Water ice under thin layer of dry soil

Basic idea: vapor diffusion through sand create a “wet layer” with liquid water films
Experimental setup

The main part — vacuum chamber

1. water ice,
2. sand containing biological sample,
3. Thermoelectric couple,
4. heating element (0.2 W power)
• **Sample structure:**

— water ice at the bottom (1 cm$^3$)
— sand with non-extremophile bacteria *Vibrio Sp.* on top.
— nutrients were added to sand: $10^{-3}$ weight fraction of M9 Standard Minimal Medium ($\text{Na}_2\text{HPO}_4$, $\text{KH}_2\text{PO}_4$, $\text{NaCl}$, $\text{NH}_4\text{Cl}$, $\text{MgSO}_4$, $\text{CaCl}_2$) and $10^{-5}$ weight fraction of glucose
Simulating of diurnal temperature cycle and sublimation

Basic idea – ice sublimation and further vapor diffusion through sand create a “wet layer” with liquid water films

- **Single diurnal experimental cycle includes:**
  - heating of the sand’s surface up to 280-300 K
  - maintaining of temperature during 4-5 hours
  - then cooling down to 200 K and maintaining of temperature during 19-20 hours.

- **Experimental run includes three diurnal cycles**

- **Average ice sublimation rate is less than 0.01 g·cm⁻²·hour⁻¹**

- **Average coefficient of the vapor diffusion (D) through the sand**  
  \[(1.55-1.65) \times 10^{-4} \text{ m}^2\text{s}^{-1}\]  
  => close to  \[D=1.74 \times 10^{-4} \text{ m}^2\text{s}^{-1}\]  
  of the accepted analogue for the martian regolith JSC Mars-1 (Chevrier, 2007).
Structure of the experimental sample after several hours of heating

- *Dry sand above*
- Weight fraction of water in the "wet" layer up to 30%.
- *Ice remnant*
Biological sample: *Vibrio Sp.*

— the wild strain of *Vibrio Sp.* obtained from the natural environment (moister soil of Baltic Sea region)

**Characteristics of the strain:**

- mesophile;
- facultative anaerobe;
- ability to survive desiccation and freezing;
- can grow in M9 Minimal Medium;
- optimal conditions for growth:
  - $5 \leq \text{pH} \leq 9$;
  - salinity $< 3\%$. 
Optimal temperature for growth: 37°C - under this temperature time period needed for doubling of cell’s population is equal to 0.5 hour. Doubling time increases with temperature lowering:

- at 15°C - 3.5 hours,
- 10°C – 4.5 hours,
- 6°C - 40 hours,
- 3°C - 96 hours

Specific properties which simplify identification of bacteria:

- resistance to ampicilin;
- change of pH from neutral to 9 at stationary phase;
- growth on chitin powder

Biological sample: *Vibrio Sp.*
Results

• We discovered the reproduction of Vibrio Sp. in “wet layer”.

• CFU number increases up to 100 times in comparison with control samples after the run with heating up to 300 K.

• CFU number increases up to 5-6 times in comparison with control samples after the run with heating up to 280 K.

• CFU number doesn’t change in comparison with control sample after the run without heating.

Main conclusion – low atmospheric pressure itself is not limiting factor for reproducing of terrestrial microorganisms in martian subsurface layer.

More critical factors are water content and temperature.
Summary

• Our experiments demonstrate the possibility of metabolic activity and reproduction of terrestrial non-extremophile bacteria in the martian-like subsurface layer at very low atmospheric pressure 0.01-0.1 mbar.

• Necessary conditions for metabolism and reproduction are the sublimation of ground ice through a thin layer of martian soil and short episodes of warm temperatures in the vapor diffusion layer.

Presence of primitive living forms in martian shallow subsurface can not be excluded in present conditions

• Remaining problems for subsurface martian life – energy and nutrients.
Conclusions

- High radioresistance could evolve in the cyclic process of sublethal irradiation followed by the recovery of population (around 100 cycles).
- Subsurface layer of Martian soil are the most plausible places in the Solar System, where such a cyclic process would be possible.
- Perhaps, the exchange of micro-organisms between Mars and Earth have happened many times. It could produce similar genome of the terrestrial and hypothetical martian microorganisms.
References

• Pavlov A.K. et al, (2006), Astrobiology, 6(6), 911-918