SURVIVAL STRATEGY OF BACTERIA IN PERMAFROST AS A MODEL IN ASTROBIOLOGY

- *Soil Science Department, Moscow State University, Moscow, Russia,
- ** Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia.
- *** Institute of molecular genetics, Russian Academy of Sciences, Moscow, Russia
Ancient Arctic and Antarctic permafrost subsoil sediments are the regarded model for solving some important issues, relevant to astrobiology:

- The main reason to consider permafrost as exobiological model
  - Earth permafrost is the most static and balanced environment;
  - Distinct cryotexture provide a closed system without any water infiltration, so we have favorable conditions for long-term cryopreservation of microorganisms in nature:
    - constant subzero temperatures,
    - thin unfrozen water films,
    - transfer of ions and gases in unfrozen water films,
    - organic- mineral complexes with immobilized microbial cells and organic macromolecules that can play protective role for life preservation

- estimation of possible preservation of microbial life in extraterrestrial habitats on cryogenic bodies.

- determination of limits of cell viability in extreme environment,
Basing on analysis of our experimental data, it is possible to consider that long-term exposure to subzero temperatures is a factor, with which only resistant microbial cells might cope in terrestrial permafrost.

The existence of diverse microorganisms in ancient permafrost is evidently not limited by psychrophily of some species, since mesophilic prokaryotes are commonly isolated from these habitats.

Abundant bacteria, found in the Earth permafrost, have adopted perhaps due to common mechanisms that ensured stability of cells, subcellular structures and genetic material for thousands and million years.

In this communication we discuss properties of permafrost bacteria (ex situ and in situ) to be useful as targets or prototypes in further searches for life signs on extraterrestrial cryogenic bodies.
Characteristics of permafrost bacteria (*ex situ* and *in situ*) to be useful in further searches for life signs on extraterrestrial cryogenic bodies

- Resistance and viability of microbial communities to strict exposure of physical and chemical factors in native permafrost sediments.

- Cytomorphological characteristics of bacteria in native populations and cells of bacterial isolates from permafrost exposed to various external stress factors.

- Structural and physiological peculiarities of experimentally obtained bacterial dormant (“spore” or cyst like) cells, that allow to:
  - reveal “portrait” resemblance with cells in native permafrost populations,
  - find forms to be responsible for survival under cryogenic conditions.
Analysis of experimental data revealed high resistance of permafrost bacterial communities *in situ* and bacterial isolates to various factors.

- **Physical and chemical factors:**
  - Radiation, including cumulative action of space factors
  - High temperature, repeating cycles of freezing and thawing
  - Oxidants, antibiotics, mercury
  - Salinity
Some examples of bacterial permafrost resistance
Resistance of bacterial communities to high temperatures

- Heating of native permafrost samples of different age and genesis at various temperatures for 3 hours decrease total cell number in permafrost samples, counted by direct microscope observation of stained bacteria only 5-10 times lower than in native samples kept at -20°C (max cell number in control samples was determined as $10^7$ cells/g)
Effect of repeating cycles of freezing and thawing on viability of bacterial communities in different permafrost sediments and in the samples of surface tundra soil.

- Samples of permafrost sediments and tundra soil were subjected to repeating cycles of freezing at –20°C in frozen camera and thawing at the room temperature (+ 23°C). After each 10, 30 and 60 cycles number of platable* cells (% of initial number after first thawing of the samples was higher in permafrost samples ( # 1,2,3) than in the samples of tundra soil ( #4)

- *platable cells- colony forming units (CFU)/g on nutrient medium

Resistant viable cells (% of initial CFU number) in native samples of permafrost and tundra soil
Resistance of bacterial communities and strains isolate from permafrost sediments to salinity

- Growth of permafrost strain of Arthrobacter sp. 24-13, isolated from the sample of frozen sediments, (age 2 mln years) in liquid TSB medium with different concentration of NaCl

- Permafrost bacteria under the conditions of concentrating the solutions upon freezing in sediments seems to be adapted to osmotic shock by accumulating intracellular “compatible” solutes – osmoprotectants, that serve both as osmo- and cryoprotectors.

- The development of mechanism of accumulation of osmoprotectants under freezing is confirmed by resistance of bacterial communities and isolates to increased concentration of NaCl in growth media
Microorganisms in ancient permafrost are very stable to high H$_2$O$_2$ concentrations.

Total cell number counted by direct microscopy method doesn’t considerably vary in 30% H$_2$O$_2$ vapor.

Introduction of H$_2$O$_2$ to growth medium decrease slightly the number of CFU/g dw in aerobic conditions. In facultative anaerobic conditions the number of CFU/g dw practically remained at the same level.
Although much has been reported about bacterial resistance in frozen habitat, we are far from a complete picture about genetic resistance of bacteria under native conservation in permafrost. Bacterial isolates from permafrost give us a unique opportunity for direct molecular comparisons between ancient and modern-day bacteria which are experience anthropogenic stress.

Antibiotic and mercury tolerance were analyzed for bacteria isolated both from Arctic and Antarctic permafrost and bacterial isolates from modern-day soil and water samples.

Bacterial strains from more than 60 samples of Arctic permafrost reinstate viability on growth media supplemented with different antibiotics. About 100 strains henceforward validated antibiotic resistance by reinoculation of individual colonies. About 30% of permafrost isolates were crosst-resistant to two or more antibiotics.
CONTENT OF MERCURY RESISTANT BACTERIA IN PERMAFROST OF DIFFERENT GENESIS AND AGE

1- alas, 5-10K*  2-edoma, 15-40 K *  3- oler,1.8-2.5 M*

*Age of permafrost sediments in years before present (YBP). K=1,000 years; M =10^6 years

Age of permafrost coincide with the period of time when the sediments stay in frozen state.

The content of mercury resistant bacteria varied within the range 0.005-2.7% that depended on the amount of mercury in sampling sites.
Bacterial strains resistant to beta-lactams, aminoglycosides, tetracycline, chloramphenicol, sulphathiazole and trimethoprim were isolated from the samples of Arctic and Antarctic permafrost sediments.
Comparative analysis of the spectrum of antibiotic and mercury resistance, genetic characteristics of both ancient and modern-day bacteria revealed similarity in the level and resistant spectrum among the strains within one systematic group of bacteria.

- The strains from both collections revealed similar mobile elements (plasmids and transposons), where resistance genes are located.

- Thus, resistance genes were spread in natural bacterial populations long before the intensive introduction of antibiotics and mercury into the environment as anthropogenic stress.

- Resulting genetic resistance in ancient bacteria point at existence of mechanisms, that may prevent the accumulation of genetic damages in cells under long term freezing in Earth permafrost sediments.

- It seems possible to extrapolate these data on probable conservation of genetic system in living forms on extraterrestrial cryogenic bodies.
Cytomorphological study of bacterial isolates

Bacteria in permafrost samples, like the cultured cells of strains isolated from these cold habitats, preserve the structural integrity and viability both in situ and after exposure of isolates to various external stresses.

- **TEM:**
  - Undamaged cell walls, nucleoids and cytoplasm
  - Most of the intact cells were represented by small forms (0.2-0.5 µm in diam).
  - Both single cells, or in conglomerates inside extracellular matrix, possibly biofilms

- **ESEM:**
  - bacterial cells of isolates at -20°C, any visible lesions or considerably shrunken forms

---

![](image1.png)

![](image2.png)
Ultrastructure of bacterial isolates under freezing and increased salinity

- TEM- cells frozen at -90°C with subsequent freeze-substituting fixation with 2% osmium tetroxide in anhydrous acetone - no obvious damage by ice crystals

- TEM- cells preserved in nutrient medium with 20% NaCl look as dormant forms like cysts with thick capsules
Unlike other living beings, microorganisms are known to be able to survive for geological time periods under conditions unfavorable for the active growth.

To endure these conditions, microorganisms developed different strategies, one of which is entering into the resting state, associated mainly with the formation of endo- and exospores, myxospores, cysts, conidia, and others.

These dormant forms differ from vegetative cells by the morphology, ultrastructure, physiology, and biochemical composition and ensure the survival of microorganisms.

It is commonly accepted now that the majority of microorganisms in nature are non-sporulating, i.e. incapable to form such specialized resting cells, but such cells with apparently simple organization are responsible for long term survival in nature including permafrost.

What is the mechanism of such survival?
It was shown that various procaryotic microorganisms, including the non-spore-forming bacteria are capable to form cystlike cells in the cycle of microbial culture development, which possessed the attributes of resting cells.

The formation of resting cystlike cells depends upon different factors but especially upon the concentration and activity of low-molecular-weight autoregulatory factors secreted by microbial cells into the surrounding medium. By chemical nature, those factors represent isomers and homologues of structurally differing alkylhydroxybenzenes (AHBs).

The mechanism of AHBs action is based on their weak physicochemical interactions with membrane lipids and biomacromolecules resulting in polycrystallization of a lipid matrix of cell membranes with changes in their functional activity and thereby dehydration of the cellular protoplast.

The above functions of AHBs were suggested to provide for inhibition of metabolic processes in a resting cell and its resistance to adverse conditions.
Ultrastructure of cells *in situ*, *ex situ* cultured in laboratory conditions under nutrient deficiency and stored at various temperatures or subjected to freezing are similar to cyst-like forms observed in model experiments and generated under special conditions by the non-spore-forming bacteria—both isolates from permafrost and type strains in microbial collections.

- Cyst-like cells were obtained under special growth conditions and controlled by specific autoregulatory system including stimulators of autolysis and autoinducers of anabiosis. They were referred to as a new type of resting form of non-spore-forming bacteria and alternative form of spore-forming bacteria.

*These forms are characterized by:*

1. Undetectable respiratory activity (an attribute of metabolic dormancy,
2. Resistance to elevated temperatures
3. Ability to resume growth under appropriate conditions
4. Low P/S ratios and high Ca/K ratios in comparison to vegetative cells as shown by X-ray microanalysis
5. In accordance with fluorescence microscopic methods it is possible to assign cyst-like cells stained with Live/Dead in green color to viable cells resistant to external stress (A—bacterial isolate from permafrost, B—view in situ)
Storage of cystlike *Bacillus cereus* cells:
Viability and thermal resistance (70 °C, 10 min)
- storage in nutrient medium at +20°C with following heating at 70°C
- storage in nutrient medium in frozen state (-20°C) with following heating at 70°C

Freezing can be considered as “selective factor” to achieve good yields of termoresistant cells and to eliminate weakly resistant cells.
Higher resistance of cyst like cells of Micrococcus luteus to heating (70 C, 10 min) and lysozyme when compared with vegetative cells.
Compatible characteristics of cyst-like cells of microbial populations *in situ* and cyst-like cells generated in model experiments (TEM observations)

- Thick cell walls, often covered by loose capsular layer,
- presence of an intact membrane,
- fine granulated cytoplasm,
- condensed nucleoid.
- “dwarf” forms

- **Bacterial cell in situ in permafrost**

- **Cyst-like cell of permafrost bacteria obtained in model experiments**

- Cells in permafrost are similar in the structure and physiological properties to dormant cystlike cells obtained in laboratory cultures of non-spore-forming bacteria, including the isolated strains.
General conclusions

- Bacteria in permafrost samples, like the cultured cells of strains isolated from these cold habitats, preserve the structural integrity and viability after exposure to various external stresses.

- Cells in permafrost are similar in the structure and physiological properties to dormant cystlike cells in laboratory cultures of non-spore-forming bacteria, including the isolated strains. Cystlike cells are responsible for survival of non-spore-formers in Earth permafrost and may be considered as targets in exobiological explorations.

- Resistance of permafrost microbial communities to heating, osmotic shock, oxidative stress, and radiation can be due to inherent stress resistance of cystlike cells augmented by additional protection from their environment especially by adsorption on organic and mineral particles.