

Microorganisms isolated from subsurface environments and their importance for astrobiology and theoretical biology

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Abstract. Objective: the article is a review of the very controversial microbial life in subsurface environments like caves, rocks, mines, deep subsurface water and springs, in very special extreme environments. Material and Methods: the methods of isolation of the bacteria and archaea from subsurface environments are discussed too and analysed. Results: the results of years of investigations showed the possibilities of adaptation to extreme environments and survival on very long periods of times, even geological eras, of some microorganisms. The inner biochemical, physical, biological and energetic mechanisms are still not elucidated, even some features were discovered. Conclusion: an extensive and intensive work of cooperation in this field of activity is required to discover the mechanisms of long term survival in extreme conditions of the subsurface microorganisms.

Key Words: subsurface environments, astrobiology, microorganisms, extreme environments, dormant state, long term survival.

Rezumat. Obiectiv: Articolul este o trecere în revistă a controversatei probleme a vieții microbiene în mediile subterane cum ar fi peșterile, minele, apele subterane și izvoarele, în medii extreme foarte speciale. Material și metodă: metodele de izolare a bacteriilor și arhaea din mediile subterane sunt discutate și analizate în lucrare. Rezultate: rezultatele multor ani de investigații arată că este posibil ca microorganismele să se adapteze la medii extreme și să supraviețuiască chiar pe perioade de timp la nivelul unor ere geologice. Mecanismul intim biochimic, fizic, biologic și energetic este în continuare o problemă de elucidat, chiar dacă unele elemente au fost identificate. Concluzii: o muncă extensivă și intensivă în cooperare, în acest domeniu, este necesară pentru descoperirea mecanismului ce permite lunga supraviețuire în condiții extreme a microorganismelor din mediul subteran.

Cuvinte cheie: medii subterane, astrobiologie, microorganismele, medii extreme, supraviețuirea pe termen lung.

1.Introduction

The Earth subsurfaces, like rocks, minerals, deep subsurface sediments, oil wells, and others were considered without life. Pedersen (2000) made a huge review of isolation cases of different isolations of microorganisms from rocks and subsurface environments estimating a total amount of organic carbon biomass belonging to subsurface microbiota of about 325 to 518 x 10¹² kg, comparing with terrestrial and marine plants life (about 562 x 10¹² kg). From that review, many other strains were isolated by traditional cultivation or by independent methods of culture according to Amann (1995). The prove of long term survival in extreme conditions can puzzle entire teoretical biology. Microorganisms have been detected in great depth in subterranean environments, such as granite, sediments, permafrost areas, and caves, rocks in gold, copper, uranium mines. According to Pedersen & Karlsson (1995), total bacterial colony forming units isolated from groundwater are from 10³ to 10⁷ cells per ml and decreased with depth. An incredible biodiversity of deep subsurface environments was demonstrated with the ability to interfere with the cycles of carbon, phosphorous, nitrogen, sulfur, manganese and other elements (Fliermans et al 1989). About 3000 strains were isolated from three boreholes in South Carolina of about more than 500 m deep.

2. Method of Work and Isolation

The methods of isolation of different microorganisms from subsurface environment varied upon the materials of the samples (water, biofilms from caves, rocks, rock salt, different minerals, and so on). Generally speaking, to isolate in good and reproducible conditions the bacteria and Archaea from rocks, sterilizing measures must be taken as soaking in strong alkaline solutions, alcohols, fire sterilization of the surface, and so on (Stan-Lotter et al 2006).

The isolation of microorganisms further include classical methods of cultivation of aliquots of the suspensions or dilutions (with sterile water, or high salt solution, depending on strain) on Petri dishes with appropriate culture media and numbering of cfus, and/or extraction of DNA and analysis by molecular biology well known methods and techniques like DNA extraction PCR amplification, determination of 16S rRNA sequences (Ward et al 1990), taxonomic tree making as described by different authors. The purified haloarchaeal PCR products of several extractions of DNA from rock salt were used for the construction of clone libraries (Radax et al 2001). In the case of ice drilling cores there are special designed sterilized device to do this job (Christner et al 2005).

Special methods of extraction of ancient DNA were also designed for ancient samples analysis (Cano & Poinar 1993). Complex genetic determination of communities' composition can be performed with DGGE (denaturing gradient gel electrophoresis) (Muyzer & Smalla 1998).

Microscopic observation in phase contrast, fluorescence and by scanning electronic microscopies were performed following specific samples preparations and they revealed great diversity of morphotypes. One of the most interesting method is the staining with BacLight LIVE DEAD kit (Molecular Probes, Eugene USA) containing the DNA stains Propidium Iodide and Syto 9, allowing the identification of live and damaged cells (Leuko et al 2004). Another visualization technique is the DAPI staining (Porter & Feig 1980).

The FISH (fluorescent in situ hybridization) analysis is an important instrument in characterization of microbial communities (Bottari et al 2006). The extraction and characterization of PFLA (Phospholipid Fatty Acids) from environments was used too for some living microbiota determinations in deep gold mines from water, biofilm and air samples (Pfiffner et al 2006).

3. Microbiota of Subsurface Environments

3.1. Caves

Caves are environments generally characterized by dark, oligotrophy (Carpa & Butiu-Keul 2009) and constant low temperature (Gounot 1999). Some caves can contain important amount of organic material from bats or provided by active water streams from the surface. The carstic formation as stalactites from the cave Grotta di Cervi, Italy, contained some microorganisms like *Streptomyces*, *Bacillus*, *Amycolatopsis*, *Arthrobacter*, *Agromyces*, *Micrococcus*, *Nocardiopsis* and *Rhodococcus* (Laiz et al 2000), being favoured by the low and constant temperature and high humidity. From an Austrian ice caves was isolated *Arthrobacter psychrophenicus*, a psychrophilic bacteria (Margesin et al 2004). The cave contains a complex of microorganisms forming some biofilms on caves walls and carst formations (Mulec 2008). The author review the problem showing that bacteria (*Pseudomonas*, *Bacillus*, *Bacterium* and some actinomycetes), fungi (*Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*), and protozoa can be isolated from caves environment. In caves, microorganisms form dots, areas of unusual colorations, corrosion, precipitation areas, structural changes of the rocks and biofilms, showing an interaction of microorganisms with minerals (Barton 2006).

3.2. Springs and Deep Subsurface Aquifers

Sponge-like network of subterranean cavities, tunnels and crackles and some underworld lakes water layers, should be an appropriate image of subsurface environments,

especially in calcareous rock limestone and sandstones. For example, the cavities and tunnels full of subterranean water of different origin emerge to the surface by springs of fresh water, thermal or mineral water.

The dimension of the subsurface water containers varied very much depending on the content and nature of rocks and minerals, water activity and debit of the area and many other parameters. Most isolates from such environments can be often retrieved from surface springs which are related with deep reservoirs. From thermal springs, located in igneous rocks coming from 500m deep, from Bad Gastein Austria, Weidler et al (2007) isolated more than 400 clones of bacteria, eg. *Proteobacteria* (α , β , γ and δ), *Bacteroidetes*, *Planctomycetes*, and archaea (Crenarchaeota). Sequences which were obtained were related to crenarchaeotic *amoA* genes (ammonia monooxygenase subunit A) and they are useful for nitrification processes (Treusch et al 2005). They are occurring in the subterranean environment and ammonia could possibly be an energy source for the resident communities (Weidler et al 2007).

The phreatic sinkhole of Zacaton (318 m deep) was explored by autonomous robot sampler (Sahl et al 2010). The microbiota communities from the deep contain especially some different Crenarchaeota. Cyanobacteria, photosynthetic sulphite oxidizers (Chlorobi) and epsilon proteobacteria were dominant in the water column samples. Deep basalt aquifer hosts a well established microbial lithotrophic community (Stevens et al 1995).

Under the Baltic Sea floor, from the ground water were discovered and isolated some microorganisms aligned with yeast genera *Rhodotorula* and *Cryptococcus* and some fungi genera, but they are over numbered by different bacterial strains (Ekendahl et al 2003).

In Australian subsurface is the Great Artesian Basin, a huge deep aquifer, from where an anaerobic thermophile, the strain *Fervidobacterium gondwanenses* was isolated (Andrews & Patel 1996).

3.3. Rocks and Minerals

Amber. The isolation of different microorganisms from rocks is a real challenge for scientists. Amber nuggets are fossilized resins of coniferous and leguminous plants, using as self defense, most of them belonging to Miocene and Cretaceous eras, containing different other biological materials (Schmidt et al 2010) entrapped inside (pollen, insects and other arthropods, yeasts, bacteria, plants remains, fungi and so on). Many microfossils of eukaryotes (in specially protists and amoebae), some cysts and spores, fungi and bacteria of Cretaceous age, recovered from amber, were reviewed by Martin-Gonzales et al (2009). DNA extracted from the samples helps to identify strains similarly with *Saccharomyces cerevisiae* and *Candida albicans* but they could not be isolated like living microorganisms. The isolation from amber of some living bacterial strain from a fossil insect (Cano & Borucki 1995), of two *Bacillus* strains (*Bacillus amyloliquifaciens* and *B. atrophaeus*) from Dominican amber (Alharbi 2008) and many sequences belonging to past microbiota (Veiga-Crespo et al 2007) prove that the long time survival and preservation of organic material is possible. All the cases of isolation and of recovery of fossil or living microorganisms, and DNA material, show that the amber is a good record of past ancient life on Earth and can provide important information on life evolution.

Rock Salt and Mines. Rock salt in the ground are formed in geological eras from Permian, Triassic and Miocene, originated from the evaporation of ancient seas and oceans and buried by diagenesis and mountain formation in about 280 to 192 M years; Permo-Triassic age for Alpine and Zechstein deposits (Holser & Kaplan 1966) were determined by deposits by sulfur-isotope analysis (ratios of $^{32}\text{S}/^{34}\text{S}$ as measured by mass spectrometry and pallinographic studies (Klaus 1974). The salt deposits from Romania are of Miocene age of about 14 M years ago. Such deposits are in all over the world as remnant salt of the ancient evaporated seas and oceans. From different such formations, isolation of viable colony forming units of microorganisms were reported, from Permian Salado formation, USA (Vreeland et al 1998), the *Halococcus salifodinae* DSM 8989,

isolated from Austrian (Denner et al 1994) and British mines at a so big distance one from another, *Halococcus dombrowski* DSM 14522 (see Figure 1) from an Austrian salt mine (Stan-Lotter et al 2002), a rod form strain *Halobacterium noricense* A1 DSM 15987 (Gruber et al 2004) and many isolates and DNA sequences (uncultured) from Polish, British and Thai mines (McGenity et al 2000; Radax et al 2001) are waiting to be described. In addition, the quantitative measurements revealed 1-2 cells/kg of salt for the British mine (Norton et al 1993) and 1.3×10^5 colony forming units (CFUs) per kg of salt in Austrian mines (Stan-Lotter et al 2000). *Hcc. salifodinae* and *Hcc. dombrowskii* have not been found yet in any hypersaline surface waters, or any location in the outside world environments.

From Miocene age rock salt, about 65 m deep horizon from the Slanic Prahova salt work (Figure 2), Romania, were isolated some strains of halophilic archaea, one of them being a *Halorubrum* strain (Fendrihan 2007, unpublished data).

Simulation experiments with haloarchaeal cells embedded in artificial halite suggested that the microorganisms can survive while enclosed in fluid inclusions (Fendrihan et al 2006).

Gold Mines. The mines from Witwatersrand Basin, ones of the deepest in the world, contains in biofilms water and air mesophilic heterotrophic bacteria, thermophiles, sulfate reducers, and metal reducers as determined by PFLA analysis and molecular genetic analysis both aerobic and anaerobic (Piffner et al 2006). A strain, named *Geobacillus thermoleovorans* was isolated too from a very deep mine gallery (Deflaun et al 2007).

Iron Mines. The iron and copper mine Kamaishii (Japan) provided to the researcher some bacterial isolates, many of them aligned with genus *Desulfotomaculum*, a gram positive sulfate reducer, and many anaerobes, but some sequences are related with aerobes *Xylophilus ampelinus* and *Acidovorax* sp. (Ishii et al 2000). The biogenic origin by precipitation of some mineral iron ores (biologically induced) was demonstrated (Akai et al 1999).

Copper Mines. Bireley (1978) identified in copper mines leaching dumps an iron oxidizer, a *Thiobacillus* like thermophilic microorganism. *Ralstonia pickettii* strain DX-T3-01 and *Sphingomonas* sp. strain DX-T3-03, respectively were isolated from a copper mine from China showing a high resistance to cadmium, copper, zinc and nickel (Xie et al 2010). The communities differ great from site type to another, for example main acidic drainage (1.5 to 3 pH) is different in composition from inside mine environments and generally members of the taxa *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Nitrospira*, *Actinobacteria* from *Acidithiobacillus*, *Leptospirillum* and others (Yin et al 2008).

Coal Mines. Some microorganisms from coal mines for example from a lignite from a Slovakian coal mine were isolated demonstrating a diverse microbial community formed by strains of bacteria like *Rhodococcus*, *Bacillus*, and fungi *Trichoderma*, *Epicoccum*, *Penicillium*, and so on (Pokorný et al 2005).

Uranium Mines. From the uranium mines bioleachate effluents samples the scientists isolated an entire microbiota of iron oxidizing bacteria like *Thiobacillus ferrooxidans*, 60 acidophilic heterotrophs and sulphur oxidizers, and some non-sulfur oxidating acidophilic heterotrophs like *Acidiphilium* genus (Bertelot et al 1997). The sulfate reducing bacteria (SRB) can be used to clean the wastes waters (Benedetto et al 2005) and to recover elements from the leachate and tailings the thorium and uranium by accumulation, using for example an *Arthrobacter* sp., US-10 strain isolated from uranium deposit (Tsuruta et al 2007).

Oil Wells. Some of the first work on the bacteria in water originated from oil wells was issued by Neave & Buswell (1926). SRB strains (by culture independent methods) related to *Desulfovibrionaceae*, *Desulfococcus* *biacutus* were detected from oil samples and water samples but genera *Desulfomonile* sp., *Desulfotomaculum* sp. and *Desulfosarcina* sp.

were detected only in water samples (Liu et al 2008). An anaerobic methane producer *Methanocalculus halotolerans* was isolated from an oil well (Olivier et al 1998).

Sediments of Oceans and Seas. Deep sediments are generally anoxic, with some thermal activity or have only low temperatures. From deep anoxic sediments with hypersaline environment from Mediterranean Sea, were isolated some *Bacillus* like strains (spores) and other related to *Alteromonas*, *Pseudomonas* and *Halomonas* growing on a very diverse organic carbon sources (Sass et al 2008). Strains of methanogenic Archaea were isolated from deep sediments like *Methanobrevibacter smithii* from sediments in natural gas fields (Mochimaru et al 2009).

Interesting formations are the ikkaite tufa columns from the Ikka Fjord, SW Greenland with a pH of 10.4 and in cold environments about 4°C containing many alkaliphile and psychrophilic microorganisms identified by molecular biological methods some of them being new strains (Schmidt et al 2006).

The stromatolites are other interesting biosedimentary formations and can be retrieving in places like Shark bay Australia in hypersaline environments beneath the ocean. From those structures a huge microbial biodiversity was identified: cyanobacteria like *Xenococcus*, *Microcoleus*, *Leptolyngbya*, *Plectonema*, *Synechococcus*, *Pleurocapsa*; bacteria from *Proeobacteria*, low CG Gram positive *Planctomycetes*, *Acidobacteria*, methanogenic Archaea – *Methanosarcinales* (Burns et al 2004) and extreme halophiles as *Halococcus hammelinensis* (Goh et al 2005). Fe-rich stromatolites are constructed by the activity of eukaryotes like *Euglena mutabilis* in acid mine drainage, being a model for ancient Precambrian stromatolites formations (Brake et al 2002).

Mud volcanoes, for example, those from Mariana Foreach, hosts many extremophilic Archaea (Euryarchaeota as well as Marine Benthic Group B Crenarchaeota) consistent with anaerobic methane oxidation and sulfate reduction (Curtis et al 2009).

3.4. Permafrost

The permafrosts are soils which stay under 0°C almost two consecutive years. They are specific to Arctic areas and the average temperature is -16°C, Siberia -11°C and in Antarctic with temperatures between -18 and -27°C (Vorobyova et al 1997). The conditions are of low temperatures, low water availability and content of natural radio nuclides (Gilichinsky et al 2008). The microorganisms isolated from those environments are methanogenic archaea like *Methanomicrobiaceae* (Ganzert et al 2007) and bacteria denitrifiers, iron and sulphate reducers (Rivkina et al 1998), aerobic and anaerobic heterotrophs. Many isolates belongs, according to Steven et al (2007), to genera *Micrococcus*, *Bacillus*, *Paenibacillus*, *Rhodococcus*, *Arthrobacter*, *Haloarcula*, and *Halobaculum*. Some of the isolates look like to be entrapped in 3 to 5 M years old layers or younger layers, like the *Actinobacteria* and *Gammaproteobacteria* isolated from a 25.000 years permafrost layer (Katayama et al 2007). Very old DNA sequences were isolated from ancient ice supposed to belong to ancient microorganisms (Biddle et al 2007). Some isolates as seen on direct scanning electron microscopy revealed dwarf forms and cyst-like cells of non-spore forming bacteria (Soina et al 2004).

3.5. Ice and Glaciers

Ice glaciers from Antarctica represent about 90% from the ice of our planet (Christner et al 2008). Ice and glaciers are found in terrestrial environments, in Polar areas, in alpine areas, on sea, or lakes. The ice contains some biological organic material like bacteria and other microorganisms, pollen and so on, initially brought by air or water currents. From ice layers of 100-200.000 years from the deep of 1500-2000m the ultramicrobacterium *Herminimonas glaciei* was isolated (Loveland-Curtze et al 2009) and from 120.000 year layers from a Greenland glacier *Chryseobacterium greenlandense* (Loveland-Curtze et al 2010). From a 750.000 year old ice layer from Tibet plateau *Pseudomonas* and *Acinetobacter* sequences (Christner et al 2003) were isolated. The ice microbial community (named SIMCO - Sea Ice Microbial Community - Hollibaugh et al

2007) is formed by algae, fungi and bacteria, which contains methanogens, sulphate reducers, aerobic chemoheterotrophs, anaerobic nitrate reducers (Skidmore et al 2000) and viruses (Deming 2007).

4. Comments on Adaptations to Extreme Environments

Generally speaking, there is much more poly-extremophilic microorganism, adapted in the same time to more than one extreme value of environmental parameters, for example, to higher/lower temperature and high pressure, or hypersaline conditions and alkaline environments and so on. In the subsurface environments can be found many types of microorganisms which developed specific adaptations according to their habitats and taxonomic group. Depending on the habitats, there are in subsurface environments, thermophiles, psychrophiles, piezophiles, halophiles, and so on (see in review Gagyipalffy & Stoian 2008), or polyextremophiles.

Cristner (2002) showed the incorporation of the protein and DNA precursors by *Arthrobacter* and *Psychrobacter* at -15°C , and the psychrophilic strain *Psychromonas ingrahami* showed a growth with a slow growth rate of 10 days generation time at -12°C (Breeze et al 2004). A hypothesis is that the tiny capillars and micro cavities can have a unfrozen liquid inside (Price 2007) staying fluid even at -20°C (Junge et al.2004) and at the interface ice-minerals forms a thin water layer (Watanabe & Mizoguchi 2002). The microbiota can survive in cracks and capillary tunnels containing a concentrated ionic solution with a lower freezing point (Price 2007).

Thermophiles from hydrothermal vents and thermal water and springs are adapted to very high temperatures, the upper limits being known as 113°C for *Pyrolobus fumarii* (Blöchl et al 1997).

There are different changes in psychrophiles: unsaturation of fatty acids by the enzyme desaturase, by anaerobic mechanism, by methyl branching, by shortening of the fatty acids chains, or sterol phospholipids ratio (Russell 2008). The process have similarities with the exposure to higher temperatures with apparitions of the HSPs playing the role of correcting the proteins coiling and the removal of the denaturated ones in condition of thermal stress at high temperatures.

The salt content can play a role too in the maintaining of structures, the extreme halophilic archaea and bacteria can be found in some hypersaline sediments and they fight with osmotic stress by Na out -K in mechanisms or by compatible solute synthesis and accumulation in their cells (Fendrihan et al 2006). The proteins including enzymes of extremophiles are practically modified to cope with the survival and activity in non-mesophilic conditions. The thermostable enzymes and proteins have strategies of alpha helix stabilisation like: amino acids substitutions and, for example, low frequency of C β branched amino acids Val, Thr, Ile; the stabilisation of folded protein by disulphide bridges, hydrogen bonds and hydrophobic interaction; stability of the bond between protein domains as oligomer formation via ion pairs network; a dense packing and increasing core hydrophobicity; low levels of Cys, Met, on surface exposed parts (Turner et al 2007).

At the cold adapted microorganisms, the strategy is to increase the flexibility of the proteins and enzymes for remaining active in low temperature by a reduction of arginine, glutamic acids and proline (salt bridge forming residues) and have a reduction of hydrophobic clusters (Grzymiski et al 2006). Cold shock proteins (Csp) and antifreeze proteins (AFP) (Russel 2008) are helping the survival in the cold environments by protection of the proteins by avoiding the formation of ice crystals inside the cells.

Other adaptation strategy include the development of a very efficient mechanism of DNA repair following radiation, desiccation and temperature stress, by complex systems of NER and MMR, the genes for it being detected in hyperthermophilic bacteria, but not in archaea (Grogan 2004).

Accumulation of some compatible solutes is a wide spreaded strategy, the trehalose biosynthetic ability is present in a various categories of microorganisms bacteria and archaea, eukaryotes as plant and animals and fungi. This compatible solute appears at different stressors like heat, cold, and osmotic stress (Avonce et al 2006). The

psychrophiles release exopolysaccharides that have a cryoprotective role at low temperatures (Deming 2007).

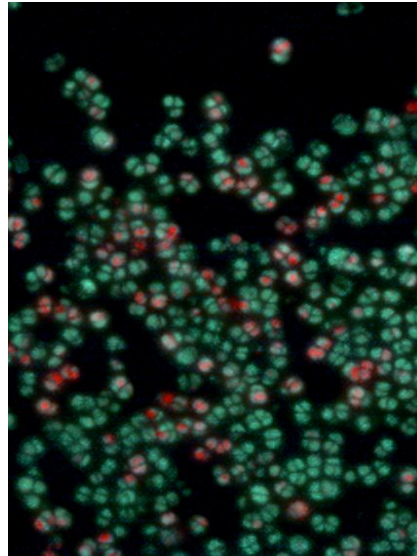


Figure 1. *Halococcus dombrowskii* strain H4, isolated from Austrian salt mine stained with BacLight LIVE DEAD kit and observed by fluorescence microscopy (photo Sergiu Fendrihan)



Figure 2. Drilling core sample from Slănic Prahova saltwork, Romania (photo Sergiu Fendrihan)

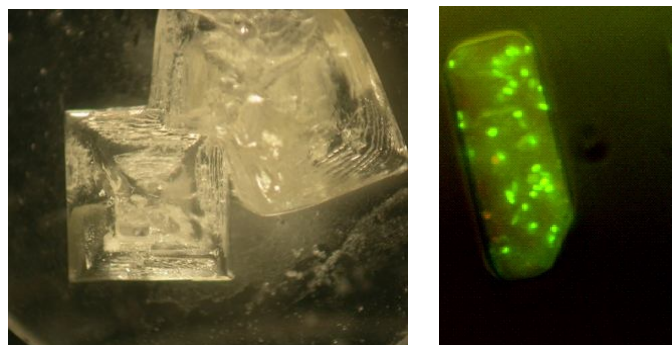


Figure 3.a) Salt crystals with haloarchaea inside;b) Fluid inclusion with *Halobacterium salinarum* strain NRC1 prestained with BacLight LIVE DEAD kit, observed by fluorescence microscopy image acquired with CCD camera (photo Sergiu Fendrihan)

The metabolism of subsurface microorganisms is very diverse and, generally speaking, many of them are chemolithotrophs (Pricop & Negrea 2009), performing sulphide reduction, Fe III, Mg reduction or other, and some others are heterotrophs. There are lots of possible metabolic pathways in order to survive in so harsh conditions. This taxonomic and metabolic diversity, show the establishment of microcenosis in those extreme habitats with producers, consumers and decomposers. In some very extreme environment can be formed H_2 driving communities formed by lithoautotrophic (chemoautotrophic) microorganisms (Nealson et al 2005). Even some wide subsurface cavities are not sterile and contain some microbiota that can be a problem for deposition of radioactive wastes being identified many metabolic types of microorganisms in the vadose zone. They can oxidise iron, manganese, magnesia, sulphur carbon and can perform some reduction reaction too, being possible to interfere with the radioactive wastes (Pedersen & Karlsson 1995).

Other strategies are the endospores for spore forming microbiota, or very slow active growth in conditions of oligotrophy and anaerobiosis, the environments selecting the microbiota with appropriate metabolic capabilities. Well known survival strategies are the endospores, but this spore-forming are in general not real extremophiles, they are maybe extreme tolerants or mesophiles which can survive as endospores for long period of time.

Some bacteria possess plasmids which confer resistance to high amount of heavy metals (Fredrickson et al 1988) or adopt other strategies, eg. using polyphosphates and polyphosphate metal transport system to cope with high amount of metal, see for example *Sulfolobus metallicus* which is able to resist to over 200 mM copper sulphate concentration (in the same time a reduction of polyphosphate accumulations from the cell was observed - Remonsellez et al 2006).

The long term viability of subsurface microorganisms in rocks like amber (Cano & Borucki 1995), halite (Stan-Lotter et al 2002, 2004) and in permafrost (Gilichinsky 2002) and ancient ice (Biddle et al 2007) is a real challenge for all the scientific community. The isolation sterile techniques (Stan-Lotter et al 2006), the isolation of the rock salt layer from outside contacts by impermeable layers (Einsele 1992), the independent isolation of the same strain from different salt deposits and in no other places at the surface (Stan-Lotter et al 1999), can be testimonials for their long term survival. The survival in fluid inclusions demonstrated by embedment of prestained cells (see Figure 3) in laboratory salt crystals (Fendrihan et al 2006), low metabolic rates, resting stages and others (Grant et al 1998) were proposed as hypothesis. The hypothesis of long term survival, combined with the prove of water and halite existence on Mars studying the Monahans meteorites (Zolensky et al 1998), SNC meteorites (Gooding 1992) and detection of a salty ocean in Europa (McCord et al 1998) showed a possibility to find some similar organisms in Mars subsurface in halite from its extinct microbiota or in other extraterrestrial environment (Stan-Lotter et al 2004). The polar areas and in specially the ice and permafrost are possible models of some extraterrestrial environments (Gilichinsky 2002). The future space and planetary exploration (Petrescu-Mag 2009) like EXOMARS mission will take advantage from all the researches in extreme environment microbiology in searching life on Mars.

Conclusions. The deep subsurface of our planets contains in a diverse range habitats microbial communities adapted by biological and morphological and biochemical mechanisms to extreme environments. The microbial communities play an important role in the biogeochemical cycles of different elements. So many cases of probable long term survival cannot be ignored, being a real challenge for modern and theoretical biology. Their survival fuel the idea of pan-spermia and the possibility to find in our space and planetary explorations similarly microorganisms in the analogs of terrestrial extreme environments. Their abilities to grow in extreme conditions and their enzymes and compounds, can be used in different biotechnological processes including bioremediation and treatment of industrial and mining wastes. There are lots of researches and experiments to prove their survival abilities, the meaning of resting stages and to isolate many other new strains from Earth extreme environments.

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