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Microbial Extremophiles at the Limits of Life

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Prokaryotic extremophiles were the first representatives of life on Earth and they are responsible for the genesis of geological structures during the evolution and creation of all currently known ecosystems. Flexibility of the genome probably allowed life to adapt to a wide spectrum of extreme environments. As a result, modern prokaryotic diversity formed in a framework of physico-chemical factors, and it is composed of: thermophilic, psychrophilic, acidophilic, alkaliphilic, halophilic, barophilic, and radioresistant species. This artificial systematics cannot reflect the multiple actions of different environmental factors since one organism could unite characteristics of several extreme-groups. In this review we show the current status of studies in all fields of extremophiles and summarize the limits of life for different species of microbial extremophiles. We also discuss the finding of extremophiles from unusual places such as soils, and briefly review recent studies of microfossils in meteorites in the context of the significance of microbial extremophiles to Astrobiology.

Keywords Extremophiles; Microbial diversity; Astrobiology; Ecology of Microorganisms; Limits of Life

INTRODUCTION

During Earth's evolution, accompanied by geophysical and climatic changes, a number of ecosystems have been formed. These ecosystems differ by the broad variety of physicochemical and biological factors composing our environment. Traditionally, pH and salinity are considered as geochemical extremes, as opposed to temperature, pressure, and radiation that are referred to as physical extremes (Van den Burg 2003). Life inhabits all possible places on Earth interacting with the environment and within itself (cross species relations). In nature it is very rare when an ecotope is inhabited by a single species. As a rule, most ecosystems contain the functionally related and evolutionarily adjusted communities (consortia and populations). In contrast to the multicellular structure of eukaryotes (tissues, organs, systems of organs, whole organism), the highest organized form of prokaryotic life in nature is presented by the benthic colonization in biofilms and microbial mats. In these complex structures all microbial cells of different species are distributed in space and time according to their functions and to physicochemical gradients that allow more effective system support, self-protection, and energy distribution. *In vitro*, of course, the most primitive organized structure for bacterial and archaeal cultures is the colony, the size, shape, color, consistency, and other specific characteristics which differ on the species or subspecies levels. In Table 1 all known types of microbial communities are shown (Pikuta et al. 2005b). Additional factors could be added to this classification Table 1: in deep-sea ecosystems (pressure), and in deep underground lithospheric ecosystems (pressure and radiation).

Currently the best-studied ecosystems are: human body (due to the medical importance), and freshwater and marine ecosystems (because of environmental concerns). For a long time, extremophiles were *terra incognita*, since the environments with aggressive parameters (compared to the human body temperature, pH, mineralization, and pressure) were considered *a priori* as a dead zone. It took time to find out that the environments with extreme physico-chemical and climatic parameters are inhabited by a wide spectrum of different microorganisms. Extremophiles were discovered in the following chronological order:

Long ago it was known that many fungi could grow in slightly acidic (pH 4–6) conditions, but the first obligately acidophilic bacterium to be described was *Acidithiobacillus ferrooxidans* (formally *Thiobacillus ferrooxidans*). Subsequently thermophilic lithotrophic acidophiles were found, and the hyperacidophilic species of the genus *Picrophilus* growing at negative pH values were described in 1996 (Schleper et al. 1996).

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TABLE 1
Known types of microbial communities

Types of communities	pH	NaCl, %(w/v)	Temperature, °C
1. Freshwater psychrophilic	5–7	0–1	<10
2. Freshwater, meso-thermal			15–40
3. Freshwater moderately thermophilic			50–60
4. Freshwater thermophilic			70–110
5. Marine psychrophilic	8	3–4	<10
6. Marine, meso-thermal			15–40
7. Marine moderately thermophilic			50–60
8. Marine thermophilic			70–120
9. Alkaliphilic psychrophilic	9–11	0–1	<10
10. Alkaliphilic mesophilic			15–40
11. Alkaliphilic moderately thermophilic			50–60
12. Alkaliphilic thermophilic			70–110
13. Haloalkaliphilic psychrophilic	9–10	3–25	<10
14. Haloalkaliphilic mesophilic			15–40
15. Haloalkaliphilic moderately thermophilic			50–60
16. Halophilic psychrophilic	8.0	3–30	<10
17. Halophilic mesophilic			15–40
18. Halophilic moderately thermophilic			50–60
19. Acidophilic mesophilic	0–4	0–2	15–40
20. Acidophilic moderately thermophilic			50–60
21. Acidophilic thermophilic			70–120

The discovery of thermophilic bacteria is generally attributed to Miquel (1888), but Brewer (1866) had already described thermophilic *Chlamydobacteriales* from the geysers of California. The modern epoch of the study of thermophilic microorganisms was triggered by the discovery of *Thermus aquaticus* (Brock and Freeze 1969), and now the maximum temperature for growth at 113°C was found for *Pyrolobus fumarii* (Blöechl et al. 1997). Another hyperthermophilic microorganism, strain 121(not validly published) that was isolated by Kashefi and Loveley (2003) survives for short periods of time at 130°C (Cowan 2004).

The first mention of the term "psychrophile" was made by Schmidt-Nielsen in 1902 for the description of bacteria capable of growth at 0°C (Morita, 1975), but Arctic diatoms had already been studied more then one hundred years ago without using this term (Van Heurck 1909). Now with the development of a special technique of cooled instruments and well equipped cold rooms it has become possible to study truly psychrophilic microorganisms. Firstly in our Astrobiology Laboratory at NASA/MSFC/NSSTC the aerobic and anaerobic bacterial growth (in pure culture) was determined at −5°C on a liquid and solid media (Hoover et al. 2002; Pikuta and Hoover 2003; Pikuta et al. 2003b), and our Russian colleges successfully cultivated aerobic bacterial cultures on solid agar media also at −5°C (Gilichinsky et al. 2005). The observations of living microorganisms *in situ* at –20°C in highly mineralized media were reported previously (Staley and Gosink 1999).

The first mentioned alkaliphile was the bacterium *Streptococcus faecalis* (Downie and Cruickshank 1928), but several years earlier an article about alkalitolerant nitrifying bacteria was published (Meek and Lipman 1922). Extreme alkaliphiles belonging to genera *Clostridium* and *Bacillus* were isolated from soils (Horikoshi and Akiba 1982), but truly alkaliphilic microorganisms belonging to separate genera such as *Natronobacterium* and *Natronococcus* were described later (Tindall et al. 1984).

Study of halophilic microorganisms has started from work with saline soils and lakes, and now the record of good growth for *Haloferax mediterranei* has been demonstrated at 30% NaCl.

The study of barophiles became possible after the development of deep-ocean submersible crafts. In the Black Smokers studies it was shown that there are microorganisms that require high pressure in addition to high temperature, and the highest known limit of life was detected at 100 MPa (Yayanos et al. 1979).

The first radioresistant bacterium *Deinococcus radiodurans* was found during the process of food conservation and storage. This bacterium could survive ionizing irradiation and other DNA-damaging assaults at doses that are lethal to all other organisms (Raj et al. 1960). Among archaea the hyperthermophilic sulfur-reducing *Thermococcus gammatolerans* that is capable of resisting 30 kGy of γ-irradiation was described comparatively recently (Edmond et al. 2003).

Anaerobiosis, as an alternative to the aerobic life, was discovered by Pasteur in his fermentation work (Pasteur 1861), but an anaerobic technique for the cultivation of obligately anaerobic microorganisms was developed much later (Hungate 1969). Among the bacteria and archaea there are many anaerobic species, and most of them are not extremophiles. For this reason an anaerobiosis is not considered as imperative to extremophilic life. However, it should be remembered that the first life forms on early Earth were anaerobic extremophiles, and therefore, this capacity is a very important issue for the logical discussion about the limits of life.

From the point of view of systematics, both eukaryotic and prokaryotic organisms represent life in extreme ecosystems, and the comparison of all taxa in complex biocoenosis of each ecosystem will provide answers to the fundamental questions of the origin, distribution, and evolution of life (direction of changes in the phenotype).

1. Acidophilic Microorganisms

It is well known that proteins denature at low pH, and from this point of view extreme acidophilic organisms represent great

potential for biotechnology and significance for the molecular and genetic research. Fish and cyanobacteria have not been found living in nature below pH 4, and plants or insects below pH 2–3 (Rothschild and Mancinelli 2001). It is exceptionally interesting that the first representative of extreme acidophiles growing near pH 0 were found to be not prokaryotic but eukaryotic microorganisms. *Cyanidium caldarium* is a red algae belonging to rhodophytes. It contains chlorophyll "a" and C-phycocyanin in its chloroplasts, and it colonizes hot, acid "soils" and waters all over the world except for Hawaii, which are geologically relatively young islands. Three other extreme acidophilic eukaryota are fungi: *Aconitium cylatium, Cephalosporium* sp., and *Trichosporon cerebriae* (Schleper et al. 1995). The green alga *Dunaliella acidophila* can survive at pH 0, but the growth maximum for this organism occurs at pH 1 (Pick 1999). However, the archaea *Picrophilus oshimae* and *P. torridus* broke all records and demonstrated that life could exist at a minimum pH of –0.2. The cells of these archaea grow within a moderately thermal regime and were found near a hydrothermal spring with solfataric gases in Japan (Schleper et al. 1996). Another extremely acidophilic microorganism *Ferroplasma acidarmanus* is eubacterium growing at pH 0 in acid mine drainage in Iron Mountain, California. This species does not have a cell wall, and the cell membrane is the only barrier between the cytoplasm and concentrated sulfuric acid with high concentrations of copper, arsenic, cadmium and zinc in the surrounding medium.

Bacteria growing at pH less then 3.0 have chemolithotrophic, chemolithomixotrophic, or chemoorganoheterotrophic metabolisms. Among acidophiles there are hyperthermophilic, moderately thermophilic, and mesophilic species. They are spore-forming and non spore-forming, aerobic, microaerophilic, or obligately anaerobic. The cell membranes of these microorganisms have a positive or negative reaction to the gram-stain, and sometimes the cell wall could be reduced to a single membrane. They are able to receive energy from hydrogen, iron, sulfur, or organic molecules. Usually participating in the process of transformation of sulfur and iron from minerals and rocks (bioleaching), acidophiles form acidic media (equilibria 1–3):

$$FeS_2 + 6Fe^{3+} + 3H_2O \leftrightarrow 7Fe^{2+} + S_2O_3^{2-} + 6H^+ \quad [1]$$

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+ \quad [2]$$

$$S_2O_3^{2-} + 2O_2 + H_2O \leftrightarrow 2SO_4^{2-} + 2H^+ \quad [3]$$

But they are also capable of the consumption of a proton that can lead to alkalinity in some mine waters (equilibrium 4):

$$2Fe^{2+} + 0.5O_2 + 2H^+ \leftrightarrow 2Fe^{3+} + H_2O \quad [4]$$

Some acidophilic microorganisms have the ability to maintain a neutral pH inside of cytoplasm, and intracellular enzymes from these organisms do not need to be adapted to extremely low pH. The internal pH of acidophiles has been measured at between 5 and 7 (Norris and Johnson 1998). However the extra cellular proteins usually exhibit adaptation to high acidity and their optimum of function occurs at low pH.

We have already discussed that microorganisms could develop an adaptation to several extremes at the same time. High temperature, low pH, and high concentration of certain metals (the term *metallophile* was used in some literature, but has not received wide acceptance) are examples of parameters that could participate in such multiple effects. In acidic environments the solubility of metals is much greater, and in many cases can reach the g l^{-1} concentrations. In cells of acidophiles the metals may accumulate above the normal physiological concentrations by the action of unspecific, constitutively expressed transport systems, whereby they become toxic. Intracellular metals can exert a toxic effect by forming coordinate bonds with anions blocking functional groups of enzymes, inhibiting transport systems, displacing essential metals from their native binding sites and disrupting cellular membrane integrity. There are five basic mechanisms (Dopson et al. 2003) that convey an increased level of cellular resistance to metals: (1) efflux of the toxic metal out of the cell; (2) enzymatic conversion; (3) intra- or extracellular sequestration; (4) exclusion by a permeability barrier; and (5) reduction in sensitivity of cellular targets. Metal resistance mechanisms of acidophiles were extensively studied for arsenic, copper, zinc, cadmium, nickel, mercury, silver, ferric iron, molybdenum, chromium, and uranium. The examples of acidophilic bacteria and archaea resistant to the toxic concentrations of As(III) are *Acidithiobacillus caldus* KU and *Sulfolobus metallicus* BC respectively. *At. ferrooxidans* strains adapted to high levels of Cu(II) could be tolerant to 800 mM (Dew et al. 1999). The toxicity of Zn(II) to *At. ferrooxidans* depends upon the growth substrate. One strain resistant to 153 mM (10 g l^{-1}) Zn(II) while growing on Fe(II) is sensitive to 92 μM (0.6 mg l^{-1}) when growing on thiosulfate (Trevors et al. 1985). Acidophilic bacteria resistant to Cd(II) include a number of *Acidiphilum* spp. and an *Acidocella* strain resistant to 700 mM Cd(II), while the maximum resistance in archaea was 10 mM in *Sulfolobus acidocaldarius* and *S. solfataricus* (Dopson et al. 2003). Adapted strains of *At. ferrooxidans* can tolerate up to 1 M Ni(II) (Dew et al. 1999). Not much data is available for mercury resistance among prokaryotes. Huber and colleagues showed that the archaeon *Metallosphaera sedula* is sensitive to mercury at concentration 5 μM (Huber et al. 1989). Guay et al. (1989) found that Ag(I) is the most toxic of the metals and ions studied: silver, cadmium, cobalt, copper, zinc, uranyl, and As(III). According to De et al. (1996) silver is toxic to *At. ferrooxidans* Fe(II) oxidation at a concentration of 0.93 μM (0.10 mg l^{-1}), however after previous silver exposure this species was tolerant to 1 mM (108 mg l^{-1}) Ag(I) (Ehrlich 1984). *Leptospirillum ferrooxidans* and *Acidiphilium cryptum* have been shown to be resistant to 500 and 300 mM Fe(III), respectively (Johnson et al. 1992). Uranium, molybdenum, and chromium also were found in acidic environments, and a number of different acidophiles were isolated from the sites containing these metals. Wong et al. (1982) showed inhibition of growth of *At. ferrooxidans* by >15 mM Cr(III). Bioleaching processes are now widely applied in bioremediation of polluted environments and for the extraction of certain

metals from low-grade ores that would not be economically viable by any other method. The calculation of the contribution of bioleaching processes for acid mine drainages showed that the ferrous iron oxidation occurs with minor participation of extremely acidophilic prokaryotes. However, moderately acidophilic iron and sulfur oxidizing bacteria appear to be more significant, particularly in waters of pH 3 (Hallberg and Johnson 2003).

It should be noted that in systematics and taxonomy of acidophilic microorganisms hyperthermophilic and moderately thermophilic species sometimes have absolutely distant and separate phylogenetic positions on the levels of genera and families. Alternatively, among psychrotolerant acidophiles the specialized taxa are absent, and no reports were made concerning truly psychrophilic acidophiles. The cases of identification of acidophiles in permafrost ecosystems were reported (Schleper et al. 1995; Johnson 1998), but only mesophilic species *Acidithiobacillus* were found as surviving unfavorable conditions (psychrotolerant strains were described without detailed characteristics of metabolism).

Enzymes from acidophiles growing at pH <2–3, such as amylases and glucoamylases are applied in the starch processing; proteases and cellulases are used in feed component; and oxidases are applied in the desulfurization of coal.

Consideration of the wide spectrum of phylogenetic diversity of microorganisms inhabiting low pH environments, the various mechanisms of resistance to metals and low $[H^+]$, and the divers cell structures represented, leads us to conclude that acidophiles (as a major group of extremophiles) did not have a monophilic ancestral root. The ability of these microorganisms to survive in aggressive acidity could possibly have resulted from widespread adaptive processes during the earliest stages of biological evolution on Earth. Our point of view concerning the origin, development, and distribution of the ancient original biota precisely in the acidic and high temperature environment as a primary ecosystem on the early Earth is supported by the fact of existence of specialized genera and families of the hyperthermophilic and moderately thermophilic acidophiles representing separate distant taxa in the systematics of prokaryotes as well as the fact of absence of obligate psychrophilic acidophilic microorganisms and correspondently non specialized taxa for them.

2. Thermophilic Microorganisms

Diversity of Thermophiles

For most known species of *Eucarya* the temperatures around 100°C usually denature proteins and nucleic acids, increase the fluidity of membranes to lethal levels, and degrade chlorophyll (above 75°C), making photosynthesis impossible. Some members of two other domains *Bacteria* and *Archaea* grow at much higher temperatures. Traditionally all thermophilic microorganisms are divided into three groups: moderately thermophilic (growth optimum at 50–60°C), thermophilic (optimum higher than 70°C), and hyperthermophilic (optimum higher than 80°C). *Archaea* consist of four phyla: Crenarchaeota (Sulfolobales-Thermoproteales branch), Euryarchaeota (extreme halophiles-Methanogens branch), "Korarchaeota" and "Nanoarchaeota." The "Korarchaeota" phylum (Barns et al. 1994; Burggraf et al. 1997) has been identified from analysis of DNA obtained from the natural environment without pure cultivation of organisms, and for this reason, its establishment remains not validated (the name of phylum written in quotations).

Another phylum of archaea, "*Nanoarchaeota*" (Huber et al. 2003) is represented by a nano-sized hyperthermophilic symbiont "*Nanoarchaeum equitans*" that does not even have a strain number, and lives obligately dependent upon *Igniococcus* strain KIN4/1. Cells of this symbiont possess an outer membrane, which is a notable exception among the Archaea (Rachel et al. 2002). This co-culture was isolated from a sample of a marine hydrothermal system near Iceland, and grows under strictly anaerobic conditions between 70 and 98°C. Minimal doubling times (70 min for *Igniococcus* strain KIN4/1, and 45 min for "*N. equitans*") was observed at 90°C, pH 6, and 2% NaCl concentration in the medium. By changing the gas mixture flow (H_2:CO_2 = 80:20) to 30 l min^{-1} to allow removal of H_2S and increase the supply of H_2, the cell density of "*N. equitans*" was raised about 10-fold, while the cell density of *Ignicoccus* remained unchanged. This means that "*N. equitans*" is sensitive to high concentrations of H_2S. It is interesting that during the late exponential growth phase of the coculture, about 80% of the "*N. equitans*" cells detach from the surface of *Igniococcus* cells and occur freely in suspension. Separated cells *"N. equitans"* could not grow on cell homogenates of *Igniococcus* strain KIN4/1, and they require a physiologically active *Igniococcus* culture. Even experiments with separation of the co-culture cells by a semipermeable membrane have failed, resulting in the conclusion that direct cell contact with the "host" microorganism was required for growth of "*N. equitans*." Furthermore, the parasitic lifestyle of "*N. equitans*" relative to *Igniococcus* strain KIN4/1 also is not excluded. This co-culture demonstrated that symbiosis or parasitism occurs not only between members of different domains, but even within the *Archaea*. In addition to the 16S RNA sequence of "*N. equitans*," three other sequences were received from the environmental high temperature biotopes (LPC33, OP9, and CU1). The sequence for LPC33 from the East Pacific Rise was identical to the sequence of "*N. equitans*," but two others differed. It was shown that the genome size of "*N. equitans*" (490 kb) is the smallest of all known *Archaea*. Continuing study will show if it is attributable to the elimination of unused genes or if it is the original shortest primitive genome. The genetics study of "*N. equitans*" showed that this species has a large number of split genes (11 protein-coding and 4 tRNA genes). The splitting of tRNA genes has not been described previously, and the splitting occurs at the position in which other archaeal tRNAs contain an intron, and joining of tRNA halves is thought to be by way of a trans-splicing reaction. Furthermore, "*N. equitans*" encodes five of the nine proteins that are conserved in all *Euryarchaea*, but they are not found in any of the *Crenarchaea* (Makarova and Koonin 2005). Subsequent discoveries of the members of "Korarchaeota" and "Nanoarchaeota"

with a validly published new species could also validate the status of these phyla.

All other phyla of the domains Bacteria and Archaea have been validated, and have well-established phylogenetic positions. In Table 2 the current taxonomy of validly published archaea is presented. The hyperthermophiles of the phylum Euryarchaeota include both methanogenic and non-methanogenic orders. Among the methanogenic orders, there are both hyperthermophilic and mesophilic species.

Among the Bacteria two deep-branched and short lineages are represented by members of the orders Thermotogales (including the genera *Thermotoga*, *Thermosipho*, and

TABLE 2
Taxonomy of validated thermophilic archaea

Phylum	Class	Order	Family	Genus
Crenarchaeota	Thermoprotei*	Thermoproteales	Thermoproteaceae	*Thermoproteus*
				Pyrobaculum
				Thermocladium
				Caldivirga
				Vulcanisaeta
			Thermofilaceae	*Thermofilum*
		"Caldispherales"	"Caldispheraceae"	*Caldisphera*
		Desulfurococcales	Desulfurococcaceae	*Desulfurococcus*
				Staphylothermus
				Aeropyrum
				Sulphobococcus
				Thermosphaera
				Igniococcus
				Thermodiscus
				Stetteria
				Acidilobus
			Pyrodictiaceae	*Pyrodictium*
				Hyperthermus
				Pyrolobus
		Sulfolobales	Sulfolobaceae	*Acidianus*
				Sulfurisphaera
				Stigiolobus
				Sulfolobus
				Metallosphaera
				Sulfurococcus
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	*Methanothermus*
	Methanococci	Methanococcales	Methanococcaceae	*Methanococcus***
	"Methanomicrobia"**	Methanomicrobiales		
		Methanosarcinales		
	Methanopyri	Methanopyrales	Methanopyraceae	*Methanopyrus*
	Halobacteria**	Halobacteriales	Halobacteriaceae	*Halloterrigena*
	Thermoplasmata	Thermoplasmatales	Thermoplasmataceae	*Thermoplasma*
			Picrophilaceae	*Picrophilus*
			"Ferroplasmataceae"	*Ferroplasma*
	Thermococci	Thermococcales	Thermococcaceae	*Thermococcus*
				Pyrococcus
				Paleococcus
	Archaeoglobi	Archaeoglobales	Archaeoglobaceae	*Archaeoglobus*
				Ferroglobus
				Geoglobus

*All orders of this class are exclusively hyperthermophilic or thermophilic (Itoh 2003).

**These classes does not contain thermophilic species (the only exception is *Haloterrigena thermotolerans* PR5^T (Montalvo-Rodriguez et al., 2000) with temperature optimum for growth at 50°C).

***Thermophilic species of the genus *Methanococcus* are: *M. thermolithotrophicus, M. jannaschii*, and *M. igneus*.

Fervidobacterium) and Aquificales (including one genus *Aquifex*) and they are all hyperthermophiles (Itoh 2003). Moderately thermophilic bacteria have enormous diversity in phylogeny, probably the result of horizontal genes transfer during the latest stages of life's evolution.

Physiology of Thermophiles

The physiology of thermophilic microorganisms has a wide diversity, and they are usually well adjusted to their biotopes (Huber and Stetter 1998). They could be primary producers of organic matter, as well as primary and secondary decomposers within the community. Chemolithoautotrophs and organotrophs with mixotrophic and heterotrophic anabolism occur among them. Some of them have respiratory catabolism and are dependent upon external electron acceptors (sulfur, iron, sulfate, nitrate, oxygen), or have fermentative metabolism. In *P. furiosus* the Embden-Meyerhof pathway was found to be modified and includes a number of novel enzymes—ADP-dependent glucokinase, ADP-dependent phosphofruktokinase, glyceraldehydes-3-phosphate ferredoxin oxidoreductase, phosphoenolpyruvate synthase, pyruvate-ferredoxin oxidoreductase, and ADP-forming acetyl-CoA synthetase (Sakuraba and Ohshima 2002). Enzymes of hyperthermophiles, besides having high-temperature stability, are usually highly stable against detergents, organic solvents, and some other chemical reagents. Relative to pH, salinity and redox potential all thermophiles could be divided into several physiological groups: extreme acidophiles, moderate acidophiles, neutrophiles, alkaliphiles, etc.). They could be aerobic and anaerobic. But it is important to note that, for deep-sea and deep underground ecosystems, the biological standard state refers to neutral pH, which is not pH 7. Only at 25°C and 1 bar pressure does the pH 7 represent neutrality, but at the pressure of water saturation and 100°C, the neutral pH is 6.13, and at 200°C, it drops to 5.64 (Amend and Teske 2005).

Moderately thermophilic species of the genus *Picrophilus* still hold the record as the most acidophilic among extreme acidophiles (see Section 1). Members of the genus *Sulfolobus* are strict aerobes growing autotrophically by oxidation of sulfur, hydrogen sulfide, or hydrogen, forming sulfuric acid or water as an end product. Members of the genus *Acidianus* are able to grow by oxidation of hydrogen, organic matter, sulfur, sulfides, and even as *A. brierlyi* by leaching of sulfidic ores.

Among moderately acidophilic and neutrophilic thermophiles there are endemic species, for example species of the genus *Methanothermus* were found only in hot springs of the Kerlingarfjöll mountains in Iceland (Stetter et al. 1981).

Thermococcus alkaliphilus is able to grow up to pH 10.5. However, in nature, alkaline hot springs are less widely distributed then acidic (at least at the bottom of the ocean).

Some hyperthermophiles appear to be highly adapted to toxic metal ions or metalloids (Holden and Adams 2003). Arsenate and selenate are generally toxic to microorganisms; however, the crenarchaeon *Pyrobaculum arsenaticum* isolated from a hot freshwater spring near Naples, Italy, grows on hydrogen and carbon dioxide as energy and carbon sources using 10 mM arsenate or 50 mM selenate as the electron acceptor. *P. islandicum* is unable reduce arsenicum or selenium, but it is capable of oxidizing hydrogen or components of peptone or yeast extract and reducing various metals such as Fe (III), U (VI), Tc(VII), Cr(VI), Co(III), and Mn(IV) at 100°C. The iron reduction in *P. islandicum* occurs by a mechanism that differs from the NADH- and cytochrome *c*-dependent process that occurs in mesophilic iron reducers such as species of *Shewanella* and *Geobacter* (Childers and Lovley 2001). *P. islandicum* does not contain *c*−type cytochromes and its NADPH is better than NADH as an electron donor for Fe(III) reductases activity (K_m = 0.04 and 3.33 mM).

Thermostable enzymes are classified into three groups (Yano and Poulos 2003) defined by their range of temperature stability: moderately thermostable (45–65°C), thermostable (65–85°C), and extremely thermostable (>85°C). Hyperthermophilic enzymes can have a higher temperature optimum than the original organism; for example amylopullulanase could have activity up to 142°C (Schuliger et al. 1993). In some literature the enzymes from thermophiles are called thermozymes (Li et al. 2005). Thermostable proteins generally show higher α–helical and β–sheet contents than mesophilic proteins (Nagi and Regan 1997), and they show a very slow unfolding rate (Vieille and Zeikus 2001), indicating that retaining the native structure in denatured conditions is anticipated. Also it was shown that thermostable proteins contain many charged and hydrophobic residues (Haney et al. 1999). Extracellular enzymes like amylases, proteases, xylanases or pullulanases, and intracellular enzymes like dehydrogenases, oxidoreductases, and DNA polymerases were isolated from *Thermotoga maritima, Pyrococcus furiosus, Thermococcus* spp., and other hyperthermophiles.

Three principle methods (i.e., large-scale studies utilizing databases, the directed evolution, and the comparative structural analyses, and protein engineering) have identified several factors that increase thermal stability. These include a decrease in loop length and a concomitant increase in secondary structure; a decrease in labile residues (such as cysteines, asparagines, and glutamines); an increase in aromatic stacking, increased hydrophobic interactions; increased metal-binding capacity; and increased oligomerization (Yano and Poulos 2003).

Chromosomes of hyperthermophiles appear to be densely packed with genes, most of which are required for essential functions. This suggests that the earliest life forms may have had small genomes (Fujiwara 2002).

3. Barophilic Microorganisms

Pressure is a key physical parameter, which has influenced the evolution and distribution of both microorganisms and macroorganisms (Bartlett 2002). Hominids evolved at an atmospheric pressure of 0.101 MPa (= 1 atmosphere = 1.013 bar), although our aquatic ancestors originated under hydrostatic pressure

(Rothschild and Mancinelli 2001). The oceans have an average depth of 3800 m and thus an average pressure of 38 MPa as well as a maximal depth of approximately 11000 m (∼110 MPa). Hydrostatic pressure increases at a rate of 10.5 kPa per meter depth, compared with 22.6 kPa per meter for lithostatic pressure. Pressure decreases with altitude, so that by 10 km above sea level, atmospheric pressure is almost a quarter of that at sea level. The boiling point of water increases with pressure, so water at the bottom of the ocean remains liquid at 400°C. Since liquid water at the Earth's surface does not normally occur above 100°C, increased pressure can increase the optimal temperature for microbial growth, but usually by only a few degrees (Pledger et al. 1994). High-pressure environments include deep lakes and the deep subsurface regions. They include Lake Baikal in Siberia, Russia, and Lake Vostok in Antarctica. Subsurface communities of microorganisms were found as deep as 3500 m below the surface (Szewzyk et al. 1994). It is predicted that the largest number of prokaryotes in the biosphere occurs under ground (Whitman et al. 1998).

Increased pressure changes the volume and compresses packaging of lipids resulting in decreased membrane fluidity. Many organisms on Earth have adapted to very high pressure, and a sudden change of pressure could be lethal. The Mariana Trench is the world's deepest sea floor at 10,898 m below sea level, yet it harbors organisms that grow at standard temperature and pressure. It is also inhabited by an obligately piezophilic species that can grow at 70 to 80 MPa, but not below 50 MPa (Kato et al. 1998).

Barophilic bacteria are defined as those displaying optimal growth at pressures more then 40 MPa, whereas barotolerant bacteria display optimal growth at pressure less then 40 MPa and can grow well at a pressure of one atmosphere. Biodiversity of deep-sea ecosystems usually divides into hyperthermophiles and psychrophiles. The bottom of the deep sea is a world exposed to extremely high pressure and low temperature (+2°C) but, in areas around hydrothermal vents, the temperature can rise to 400°C. The first barophilic bacterium was isolated from a depth of 10,500 m in 1979 (Yayanos et al. 1979). This isolate grew at a pressure more than 100 MPa at 2°C and more than 40 MPa at temperatures exceeding 100°C. Takami et al. (1997) isolated thousands of microorganisms from the mud samples collected from the Mariana Trench. The agar plates in this investigation were incubated at 4–75°C at atmospheric pressure or at 100 MPa for 1–4 weeks. The biodiversity was found to be composed of: actinomycetes, fungi, non-extremophilic bacteria and various extremophiles such as alkaliphiles, thermophiles, three barophiles, and psychrophiles. Kato with coauthors (Kato et al. 1995; Kato et al. 1996) have reported several high pressure adapted bacteria isolated from samples of deep-sea sediment from a depth of 2500–6500 m. Most of these isolates had barophilic and psychrophilic physiology. At atmospheric pressure strain DB6705 grew only at 4°C, but not above 10°C. At conditions of high pressure (>50 MPa) this strain was able to grow better at high temperature than at the lower temperature. Another extremely barophilic bacteria from the Mariana Trench could grow at pressure no less than 50 MPa. The isolates obtained by Yayanos became more barophilic at higher temperatures (Yayanos 1986).

Hyperthermophilic barophiles *Thermococcus profundus* and *Pyrococcus horikoshii* were isolated from bacterial mat samples and hot fluid samples of hydrothermal vents in the Mid-Okinawa Trough at a depth of 1395 m (Kobayashi et al. 1994; Gonzales et al. 1998). Another archaeon *T. peptonophilus* (strains OG1 and SM-2) were isolated from hot fluid samples of hydrothermal vents in the Izu-Bonin forearc at a depth of 1380 m and in the South Mariana Trench at a depth of 1484 m respectively (Gonzales et al. 1995). Maximal growth rates of *T. peptonophilus* were observed at 30–45 MPa whereas growth at 60 MPa was slower; the optimal growth temperature shifted from 85°C at 30 MPa to 90–95°C at 45 MPa. The maximum growth rate of *P. horikoshii* was observed at 95°C under pressure of 0.1–15 MPa, and at 100°C at a pressure of 30 MPa, with a doubling time of 35 min.

Some calculations indicate that all oceanic water passes through the basement rocks every 30 million years. Large parts of the new ocean floor consist of pillow lavas with amorphic volcanic glass on the pillow surface. The microscopy and culturing evidently suggest that microorganisms were involved in the weathering of this volcanic glass (Fisk et al. 1998). Channels, cavities, and holes in the micrometer range penetrate the glass and the tips of the tunnels commonly stain with nucleic acid specific stains, suggesting the presence of microorganisms. The circulation of salt-water through the basement and the weathering activities of barophilic microorganisms in the flow paths therefore may have a significant impact on the composition of saltwater (Thorseth et al. 1995).

The intraterrestrial microorganisms dwell in the pores of consolidated sediments and coarser, unconsolidated material, in fractures in hard rock and in fluid inclusions. Almost all very deep environments are anaerobic, with the exception of places where radioactivity may cause radiolysis of water, producing hydrogen and oxygen (Pedersen 2000). The research of continental sedimentary rocks shows that living microorganisms are present and active down to the deepest levels studied (∼3000 m). Large phylogenetically and culturally diverse communities of Archaea and Bacteria in North American deep subsurface continental sediments have been shown as well as other investigated underground sites in Africa and Scandinavia (Pederson et al. 1996; Chandler et al. 1997; Crozier et al. 1999). Successful isolations of thermophiles have been performed from samples of subsea floor and continental oil reservoirs, indicating that Bacteria and Archaea (*Archaeoglobus fulgidus, Thermodesulforhabdus norvegucus, Thermotoga subterranea*) inhabit deep hot oil reservoirs (Beeder et al. 1994; Beeder et al. 1995; Jeanthon et al. 1995).

Concerning the cultivation of microorganisms from samples of super deep wells it is important to note that the borehole windows into super deep environments are still very few and

none of them have been drilled with a microbiological purpose. The deepest well SG-3, 12262 m deep, occurs in the Pechenga-Zapolyarny area of Kola Peninsula, Russia. There are reports about the successful isolation of microorganisms from such ecosystems, but none of them had good negative controls that precluded contamination (Pedersen 2000).

Igneous rocks are the predominant solid constituents of the earth, formed through cooling of molten or partly molten material at or beneath the Earth's surface. The studies in the Swedish long-term nuclear waste disposal research program on subterranean microbiology revealed previously unknown microbial ecosystems in igneous rock aquifers at depths exceeding 1000 m (Pedersen and Ekendahl 1990). The study of subterranean microbial diversity led to isolation of pure cultures of methanogens, methanotrophs, and sulfate-reducing bacteria (Kotelnikova and Pedersen 1998; Kotelnikova et al. 1998; Motamedi and Pedersen 1998; Kalyuzhnaya et al. 1999).

Barophilic enzymes, currently referred to as piezostable enzymes, are stable at high pressures and have been isolated from a wide variety of organisms, most of which are either thermophilic or psychrophilic and have optimal growth at above one atmosphere (Abe and Horikoshi 2001).

New findings from deep-sea ecosystems have important applications in medicine and biotechnology. Mesophilic *Vibrio diabolicus* isolated from a vent sample, secretes an innovative exopolysaccharide of potential medical interest for its chemical resemblance to heparin, which delays the onset of blood clotting (Raguenes et al. 1997). Another new mesophilic vent strain of *Pseudomonas aeruginosa* is remarkable for its tolerance to high concentrations of cadmium (up to 5 mM). It not only tolerates but completely (>99%) removes the cadmium from solution by precipitation onto the cell wall, making it a prime candidate for applications in heavy-metal recovery and environmental remediation (Wang et al. 1997).

It was reported that a high-pressure-regulated system for gene expression was found not only in deep-sea-adapted microorganisms, but also in bacteria growing at atmospheric pressure (Sato et al. 1995; Welch and Bartlett 1996). These results suggest that the systems developed in the high-pressure environment may be conserved in organisms growing at atmospheric pressure, possibly indicating that life emerged from the deep-sea environment a long time ago. Pressure is the only stressor known to simultaneously induce many heat- and cold-shock proteins, sets of proteins, which are induced following exposure to opposing thermal regimes. The major pressure-inducible protein is an unknown small highly basic protein. The heat-shock proteins may also be induced in piezophiles upon decompression. A stress protein showing similarity to heat-shock proteins, was found to be induced in the deep-sea piezophilic hyperthermophile *Thermococcus barophilus* at cultivation under atmospheric pressure (Marteinsson et al. 1999). Other interesting effects of high pressure on the structure of biological cells have been discussed in a previous review (Bartlett 2002).

4. Psychrophilic Microorganisms

Hooker observed living algae associated with ice in 1840. In 1887 Forster was the first to report microorganisms growing well at 0°C that were isolated from fish (Hoyoux et al. 2004). The first mention of the term "psychrophile" (cold loving) was made by Schmidt-Nielsen in 1902 in describing bacteria capable of growing at 0°C. Later the term was also used to refer to a number of species of eukaryotic organisms; including yeasts, diatoms, algae, lichens, mosses, insects, and fish. The current biological definition for psychrophilic microorganisms is the ability to grow at low and subzero temperatures, and the inability to grow with the absence of metabolic activity at a temperature higher than 15°C. True psychrophilic microorganisms undergo cell lysis at room temperature, and their enzymes and proteins are very sensitive and destabilize rapidly without maintenance in ice. Psychrotolerant microorganisms can have high metabolic activity and be capable of growth with a prolonged lag phase at subzero and low temperatures, but unlike psychrophiles they do not die at room temperature and they usually have an optimum growth in the range for mesophilic microorganisms. Here we specially ignore the term "psychrotrophic," since the exact translation means "eating cold," which is an inappropriate due to the tolerance of cold, but not trophy.

In Northern geographical regions, the surfaces of large snow fields are sometimes colored pink or red, because of the growth of snow algae, such as a species of *Chlamydomonas nivalis*, which produce brilliant red spores. These organisms alter the albedo of the snow to induce a snowmelt and increase the availability of liquid water for other psychrophilic microorganisms. Some diatoms and other psychrophiles produce extra-cellular enzymes and ice-active substances that lead to the pitting of ice and melting the surrounding ice making the liquid water available, which is necessary for metabolic activity (Raymond et al. 1994). It is now known that most (if not all) Antarctic Sea-Ice diatoms are able to synthesize these large macromolecular substances that appear to be glycoproteins (Raymond 2000). Among higher evolved living creatures, as for example some polar fish, the production of glycerol serves as the protection against freezing (Raymond 1995). The synthesis of such cryoprotectants leads to freeze avoidance. However, some organisms have developed mechanisms of freeze tolerance, involving drastic metabolic modifications during frost hardening, and resistance to membrane damage, solute concentration, and dehydration accompanying ice crystallization (Franks 1985).

Cryoprotectants such as glycerol or DMSO are water-miscible liquids, and they penetrate the cell and protect it from freezing by reducing the severity of dehydration effects and preventing the formation of ice crystals. In microbiological laboratories, the lyophilization (dry freezing) is a traditional way of preserving microbial cultures under vacuum at very low temperatures (–70 to –196°C) and for long periods of time, demonstrating that under specific conditions biological cells are indeed capable of surviving deep freezing, and this feature probably is universal for life.

Ecosystems and Microbial Diversity of Psychrophiles

The ecosystems of Earth with permanently low temperatures include the regions of the Arctic and Antarctic with polar ice sheets, glaciers and permafrost, the snow-caps and glaciers of high mountains, and the deep water and marine sediments of the oceans. In the polar regions, enormous areas of permafrost are formed. Permafrost is defined as frozen ground with thermal conditions in which soils and sediments remain at or below 0°C for two or more years in succession (Wagner et al. 2001). The terrestrial permafrost, which underlies more than 20% of the world's land area, is above all controlled by climatic factors and characterized by extreme terrain conditions and landforms. On Earth the thickness of the permafrost can reach several hundreds of meters, e.g., it is ~600 to 800 m thick in East Siberia (Central Yakutia). During a relatively short period of the polar summer, a thin surface zone (active region) of the permafrost sediments undergoes thawing.

The largest low-temperature ecosystem on Earth is the deep sea floor since oceans cover three quarters of the surface of the planet. The psychrophiles that inhabit this global-scale ecosystem (with a constant temperature of 4°C below a depth of 1,000 m) are true extremophiles as they are adapted not only to low temperatures, but also to further environmental constraints (Feller and Gerday 2003). In the ocean depths and in sediments they are faced with extremely high pressures, and therefore must be piezo-psychrophiles (or baro-psychrophiles). The microbial communities that are found in sea ice, which comprise bacteria, algae, fungi, and protozoa, are exposed to salt concentrations of several molar in brine veins at –20°C, and are therefore halopsychrophiles (Staley and Gosink 1999). On the snow surface of glaciers and polar caps, psychrophiles are exposed to strong ultraviolet radiation (Carpenter et al. 2000). The endolithic microbial communities that are found in rocks of the Antarctic dry deserts, which comprise lichens, yeasts, cyanobacteria and heterotrophic bacteria, survive low water and nutrient availability (Friedmann 1982). In alpine caves and cracks, microorganisms also evolve in a poor environment in the absence of light (troglopsychrophiles).

The physiological and phylogenetic diversity of psychrophilic and psychrotolerant microorganisms that were detected and isolated from cold ecosystems is very broad. They include many eukarya, almost all main physiological groups of eubacteria, and some archaea. Diatoms and other algae, cyanobacteria, fungi and yeast were also commonly reported in cold environments. Active anaerobes that have a truly psychrophilic nature occur in the following physiological groups: fermentative bacteria, methanogens, acetogens, sulfate-reducers, iron-reducers, and nitrate-reducers. In the last decade more attention has been paid to the search for psychrophilic strains of methanogens, since many (if not most) of methanogenic habitats occurred in cold climates (swamps of Siberia in Russia and of Canada). Also methane-oxidizing aerobic bacteria were found in the cold swamp water of the Arctic region (Omelchenko et al. 1993; Trotsenko and Khmelenina 2002). The first Gram-positive sulfate-reducing bacterium from Antarctica *Desulfotomaculum antarcticum* strain No.64 was isolated in 1968 (Iizuka et al. 1969). However, truly psychrophilic Gram-negative sulfate-reducers capable of growth at –1.8°C and within optima of growth at 7–10°C were isolated only in 1999 from permanently cold marine sediments off the coast of Svalbard (Knoblauch et al. 1999).

The producers (or synthetics) of organic compounds in cold environmental ecosystems includes not only photosynthetic (cyanobacteria, diatoms, green and brown algae), but also chemolithotrophic bacteria. Unique photosynthetic processes were found on the deep sea floor near black-smoker hydrothermal vents (Van Dover et al. 1996; Kolber et al. 2001); bacteria that could perform photosynthesis by using infrared wavelengths from high-temperature vents. These bacteria could switch photosynthesis on and off and are photoheterotrophic (require organic compounds for anabolism). These bacteria are not psychrophilic, but they could provide a primary biomass for the decomposition by organotrophic truly psychrophilic species that inhabit the deep-sea floor.

Mechanisms of Adaptation to Low Temperatures

Every microorganism is adapted for optimum functioning in natural ecological environments as is reflected in its physiological characteristics: range and optima of temperature, pH, salinity, etc. Any extreme change in environmental conditions from the optimum inflicts a stress on an organism. The intensity and time duration of the change usually determines whether the organism is killed, ceases growth, or has an increased lag phase of growth and reduced biomass yield (Ray 1986; Russel et al. 1995). Most bacteria are able to tolerate small changes in an environmental parameter and can adapt over the time scale of minutes, hours, or days. Microorganisms could perform this by both yielding to the stress conditions and making suitable provisions for survival or attempting to resist the stress. For most microorganisms, this tolerance can be pushed to maximum limits if the cell is provided with a sufficient opportunity to sense and adapt to the deteriorating environment. Psychrophiles have adapted their lifestyles to prefer (and sometimes to require) these extreme environments. Changes in environmental conditions away from the optimal value can cause the induction of many elaborate stress responses. These strategies are generally directed at survival rather than growth. According to recent reviews, the mechanisms of adaptation to cold temperatures could be connected with the changing of capacities of proteins (more flexible structural and conformation changes), increasing the fluidity of membranes by the changing of the unsaturation degree of fatty acids, modifications to *ante-iso-*/*iso-* branching patterns, and by shortening in the fatty acid chain length. Also, the synthesis of antifreeze glycoproteins and peptides can further depress the freezing point of cellular water.

Unsaturation of fatty acid chains is the change that is most commonly found to occur when the temperature is reduced. This increases the fluidity of the membrane because unsaturated fatty

acid groups create more disturbances to the membrane than saturated chains and it is achieved by desaturases situated in the membrane itself and thus are able to react quickly. Also, the average fatty acid chain length may be shortened, which would have the effect of increasing the fluidity of the cell membrane. After a fall in temperature, an increase in the amount and/or kind of branched fatty acids may occur. Sometimes, there may be a reduction in the proportion of cyclic fatty acids and thus an increase in mono-unsaturated straight chain fatty acids.

Sudden decreases in temperature initiates specific alterations in gene expression—cold-shock response. It involves the induction and the synthesis of cold-shock proteins. Cold shock response can involve the expression of up to 50 different cold shock proteins, depending on the species, as well as the rate and extent of temperature drop. There are two groups of proteins produced at the gene expression during cold shock response. Cold shock proteins (Csps) are synthesized at low temperature, and the larger the severity of the shock, the more Csps are produced. A second group of cold-induced proteins, the cold acclimation proteins (Caps) has also been described that are comparable to Csps and that are continuously synthesized during prolonged growth at low temperatures, and differentiate psychrophiles from mesophiles. Cold shock proteins can stabilize mRNA and re-initiate protein production. Others are also linked to maintaining the fluidity of the membrane such as inducible desaturases.

The regulation of Csp synthesis is multifactorial, and controlled at the levels of transcription and translation, as well as mRNA and protein stabilities. The main function of Csp are in the regulation of cellular protein synthesis, at the level of transcription as well as the initiation of translation, and in mRNA folding, acting as a chaperone preventing the formation of an mRNA secondary structure. The cellular function of acclimation proteins is still not well understood.

Other adaptive strategies developed by psychrophilic organisms involve the regulation of ion channel permeability, seasonal dormancy and microtubule polymerization (Detrich et al. 2000). The key adaptive strategy of psychrophiles is the modification of enzyme kinetics, allowing the emergence of metabolic rates compatible to life at low temperatures. The psychrophilic enzymes could be highly active at low temperatures and could be 10 times higher than that of their mesophilic homologues.

5. Halophilic Microorganisms

Halophilic microorganisms require salt in media for growth. Halotolerant microorganisms are not dependent upon the salts in growth media but able to tolerate up to 15% salinity. Halophiles are found in each of the three taxonomic domains: Archaea, Bacteria, and Eukarya. The metabolic diversity of halophiles is very wide: oxygenic and anoxygenic phototrophs, aerobic heterotrophs, fermenters, denitrifiers, sulfate-reducers, and methanogens (Oren 2002). With increasing salinity the diversity of metabolic types decreases dramatically. The maximal salinity limit, at which each dissimilatory process occurs, is correlated with the amount of energy generated and energetic cost of osmotic adaptation in environment.

Halobacteria include red-pigmented extremely halophilic archaea, members of the *Halobacteriaceae* family, the only family in the Halobacteriales order. Most halobacteria require 1.5 M NaCl both to grow and maintain the structural integrity of the cells. Halobacteria can be differentiated from halophilic bacteria on the basis of their archaeal characteristics, in particular the presence of ether-linked lipids. Most halobacteria are colored red or orange in cell vesicle due to the presence of C-50 carotenoids, but some strains are colorless, and those with gas vesicles form opaque, white, or pink colonies. A purple hue may be seen in halobacteria that form the bacteriorhodopsin-containing purple membrane. Halobacteria exhibit features characteristic of the archaea, including eukaryotic-like transcription and translation mechanisms, ether-linked lipids and like some bacteria, a cell wall S-layer composed of glycoprotein. A unique feature of halobacteria is the purple membrane, specialized regions of the cell membrane that contain a two-dimensional crystalline lattice of a chromoprotein, bacteriorhodopsin. Bacteriorhodopsin contains a protein moiety (bacterioopsin) and covalent bound chromophore (retinal), and it acts as a light-dependent transmembrane proton pump. The membrane potential generated can be used to drive ATP synthesis and support a period of phototrophic growth. Bacteriorhodopsin is induced by low oxygen tension and high light intensity. Also, halobacteria contain large quantities of red-orange carotenoids, which have been shown to be necessary for stimulating an active photorepair system to repair thymine dimmers resulting from ultraviolet radiation. The most abundant carotenoids are C-50 bacterioruberins, although smaller amounts of biosynthetic intermediates such as β-carotene and lycopene are also present. Retinal is produced by oxidative cleavage of β-carotene, a step that requires molecular oxygen. Several retinal proteins, in addition to bacteriorhodopsin, are also synthesized in halobacteria, including halorhodopsin, which is an inwardly directed light-driven chloride pump, and two sensory rhodopsins that mediate the phototactic response (swimming towards green light and away from blue and ultraviolet light). Like many aquatic bacteria, halobacteria produce buoyant gas vesicles that are hollow proteinaceous structures surrounding a gas filled space. The function of gas vesicles is to enable the cells to float to the more oxygenated surface layers since halobacteria are primarily aerobic and live in concentrated brines in which the solubility of molecular oxygen is low (especially at high temperatures). This also increases the availability of light for purple membrane-mediated photophosphorylation (Joo and Kim 2005).

Adaptation to the Hypersaline Environment

There are two fundamentally different strategies within the microbial world that enable microorganisms to cope with the osmotic stress inherent to the presence of high salt concentrations. In the first strategy, cells maintain high intracellular salt

concentrations, osmotically at least equivalent to the external concentrations (the "salt-in" strategy), in this case all intracellular systems should be adapted to the presence of high salt concentrations. In the second strategy, the cells maintain low salt concentrations within their cytoplasm (the "compatible-solute" strategy). In this case the osmotic pressure of the medium is balanced by organic compatible solutes, and here no special adaptation of the intracellular systems is required (Oren 1999). Microorganisms that balance the osmotic pressure of the medium with high concentrations of organic osmotic solutes also have to expend energy in ion pumps to keep intracellular ionic concentrations low and to counteract the diffusion of inorganic salts through their membranes. Na^+/H^+ antiporters have been characterized from moderately halophilic bacteria such as *Salinivibrio costicola* that mainly use organic solutes. These antiporters play a key role in keeping the intracellular Na^+ concentrations low. The activity of the Na^+/H^+ antiporters in the cytoplasmic membrane of halotolerant alga *Dunaliella salina* have been increased at the cultivation of cells in higher NaCl concentrations of medium. The need for Na^+ pumping thus may increase the energy cost of the "compatible-solutes" strategy beyond that of the synthesis of the osmolytic compounds only. The "salt-in" strategy is applied in two phylogenetically unrelated groups: aerobic extremely halophilic archaea of the order Halobacteriales and anaerobic halophilic bacteria of the order Haloanaerobiales. No organic osmotic solutes have been found in representatives of these groups, and most studies report that the intracellular ionic concentrations are similar to those of the surrounding media. The single exception to these observations is *Desulfovibrio halophilus* that could accumulate salt inside of cytoplasm and at the same time it could provide the osmotic balance by trehalose and glycine (Welsh et al. 1996). The intracellular salt composition is usually represented by molar concentrations of KCl.

The energetically cheaper salt-in option requires extensive adaptations of the intracellular machinery, and these can be achieved only in a long and complex evolutionary process. This option is used by a few specialized groups of prokaryotes only. All other organisms use organic osmotic solutes, which are expensive to produce (Oren 1999). When organic solutes are accumulated, there is a clear preference at the highest salinities for the solutions with smaller molecules, which are energetically easier to produce.

A survey of the halophilic microorganisms shows that not all known metabolic types function in the highly mineralized environments. While oxygenic and anoxygenic photosynthesis, aerobic respiration, fermentation, and denitrification can occur at or close to NaCl saturation, other physiological groups of microorganisms have never been shown to thrive at high salinities. Autotrophic methanogens, aceticlastic methanogens, dissimilatory sulfate-reducers with complete oxidation, autotrophic ammonia and nitrite oxidizers have not been found at salinities above 15%. Only one halophilic autotrophic sulfur oxidizer *Halothiobacillus halophilus* (formally *Thiobacillus halophilus*) has been isolated in pure culture; this organism oxidizes thiosulfate, tetrationate, and elemental sulfur in media with up to 24% NaCl (optimum is at 5–6 % NaCl).

In most cases a good correlation exists between the upper salt concentration, at which the different microbial processes have been shown *in situ* to occur and the ability of the corresponding microbial pure cultures to grow at high salt concentrations *in vitro*. Most microorganisms that produce organic osmolytes are also able to take up such solutes from the environment when it is available, thereby reducing the energy cost of life at high salt concentrations. Such a situation could occur in complex microbial communities where different metabolic types of microorganisms coexist, some of which may produce and excrete the osmolytic molecules in the environment. The inclusion of different kinds of osmolytes in the enrichment media for isolation of new halophiles may be recommended. That may explain why the "missing" physiological groups of halophiles remain undiscovered.

Usual proteins at high salt concentrations are in general destabilized by enhanced hydrophobic interactions. Halophilic proteins have evolved specific mechanisms that allow them to be both stable and soluble in a high KCl cytoplasmic concentration. Halophilic proteins have specific structural elements to fold into proper native form at high salt concentrations. Since all soluble halophilic enzymes have a highly negative surface charge and once folded properly their flexibility may be achieved by the repulsion forces between close charges. The instability caused by the high surface charge should somehow be compensated to avoid unfolding and it is believed that the role of high salt concentrations is to shield this high surface charge. All halophilic proteins are highly negatively charged with hydrated carboxyl groups, which maintain the solubility of proteins at high salt concentrations. The electrostatic repulsion offsets destabilization from the hydrophobic effect enhanced by salt. In the case of malate dehydrogenase (hMDH) from *Haloarcula marismortui* whose three dimensional structure has been determined by X-ray crystallography, the requirement for high NaCl or KCl concentrations for the stabilization can simply be explained by a specific low affinity binding of salt ions to the folded protein. Therefore, molar amounts of salt are necessary to saturate these binding sites.

The osmolytes that allow halophiles and halotolerant microorganisms maintain their active physiology in a highly saline environment (the "compatible-solute" strategy), were comprehensively reviewed by Roberts (2005).

Some of the extremely halophilic archaea produce bacteriocins called halocins; they inhibit a broad spectrum of bacteria. Bacteriocidal action of halocins includes inhibition of transcription, translation, Na^+/H^+ antiporer, DNA and RNA nuclease activity, pore formation, bacteriolysis, and disruption of cellular membranes.

Recently it was shown that within Halobacteriaceae there are many species capable of *p*-hydroxybenzoic acid degradation. The 44 new halophilic archaea were found to be able to grow in

0.4 mM *p*-hydroxybenzoic acid as the sole carbon and energy source (Cuadros-Orellana et al. 2006).

Due to the biotechnological importance of new molecules from halophiles, the intensive work with isolation of new strains from hyper-saline ecosystems continues. One hundred and sixty-five halophilic archaea were isolated from three different types of hypersaline lakes (Erliannor, Shangmatala, and Xilin soda lake) in Inner Mongolia (Pan et al. 2006). During the study of prokaryotic biodiversity in El Golea salt lake, in Algerian Sahara, extreme halophilic microorganisms were isolated from a site containing 290 g l^{-1} NaCl (Hacčne et al. 2004). The investigation of halophilic archaea in the crystallizers of Adriatic Sečovlje salterns by using gene fragments encoding 16S rRNA and bacteriorhodopsin as molecular markers resulted in 180 clones that were screened, and as a result 15 different 16SrRNA and 10 different bacteriorhodopsin phylotypes were detected (Pašić et al. 2005). Archaeal diversity along a transient soil salinity gradient at Salt Spring in British Columbia, Canada, was investigated with cultivation-independent approach (Walsh et al. 2005).

The biotechnological application of halophiles is continuously increasing. Halophilic microorganisms produce stable enzymes (DNAses, lipases, amylases, gelatinizes, and proteases) capable of functioning under high concentrations of salt, which have precipitated or denatured most known proteins, and most of these enzymes inactivated and denatured at NaCl concentrations below 1M. Compatible solutes now used as stabilizers of enzymes, DNA, membranes, and whole cells. Ectoine for example, stabilize polymerase chain reaction. Trehalose is used as a cryoprotectant in freeze-drying processes of biomolecules. Biosurfactants and halophilic exopolysaccharides enhance the remediation of oil-contaminated soil and water. Ether-linked lipids from archaeal halophiles have a high chemical stability and resistance against esterases and thus a higher survival rate than liposomes based on fatty acid derivatives. Halophiles used in bioremediation of oil-polluted saline ecosystems. Some halophiles are used for the bioremediation of pollutants by halogenated organic compounds. *Lactobacillus plantarum* is used for the fermentation of pickles and sauerkraut. *Anabaena cylindrica* is used in amelioration of soil salinity during crop growth.

The significance of halophiles for paleontology is of great concern as it has been reported that some halophiles were found alive in Permian, Jurassic, or even more ancient salt crystals as has been reviewed by McGenity et al. (2000). Also halophiles are important to the question of possible biodeterioration of containers that store toxic and nuclear waste, buried in deep underground salt caves.

6. Alkaliphilic Microorganisms

Alkaliphilic microorganisms are microorganisms that require a growth media with pH higher than 8.0, and cannot grow at neutral pH. There are some species that have two optima pH in dependence upon growth conditions, particularly nutrition. The most alkaliphilic microorganisms are cyanobacteria that are capable of growth at pH 12–13. Alkalitolerant organisms have optimal pH for growth at neutral pH, but are able to grow at pH 10–11. According to Horikoshi (1999) all alkaliphiles are divided on two main physiological groups of microorganisms: alkaliphiles and haloalkaliphiles. Haloalkaliphiles require high pH and they also require the presence of NaCl in the growth media. Some of the alkaliphiles and haloalkaliphiles are obligately dependent upon CO_3^{2-} ions, especially microorganisms isolated from soda lakes.

It is interesting that alkaliphilic microorganisms could be isolated from neutral or even acidic soils and feces. Two anaerobes *Anoxybacillus pushchinoensis* and *Desulfotomaculum alkaliphilum* were isolated from manure with neutral pH (Pikuta et al. 2000a, 2000b). Historically aerobic alkaliphiles were primarily isolated on media with high pH adjusted by NaOH solution. Later, after the study of the chemical composition of highly mineralized soda lakes the media for isolation of truly alkaliphilic microorganisms were based on the applications of $NaCO_3/NaHCO_3$ buffers. With the development of anaerobic techniques, additional reducers (sulfide, thiosulfate, etc.) were applied for the isolation of alkaliphilic anaerobes. Just as it was for extreme halophiles, the application of highly mineralized media (in case for alkaliphiles it was carbonate/di-carbonate composition) led to the discovery of numerous phylogenetically distant new genera and families (Zavarzin et al. 1999; Zavarzin and Zhilina 2000).

The first alkaliphilic strain *Streptococcus faecalis* was isolated in 1928 (Downie and Cruickshank 1928), but much earlier the communications about alkalitolerant nitrifying bacteria were reported (Meek and Lipman 1922). The works of Horikoshi showed the presence of extreme alkaliphiles (*Clostridium* and *Bacillus*) in soils with neutral pH. In soda lakes the dominant microorganisms are cyanobacteria including following genera: *Spirulina, Chroococcidiopsis, Nostoc, Cyanospira, Gloeocapsa, Nodularia, Synechococcus, Synechocystis, Calothrix, Scytonema, Anabaena, Lamprocystis, Thiocapsa, Thiocystis, Chromatium, Amoebobacter, Thiospirillum, Rhodobacter, Ectothiorhodospira, Chamaesiphon, Oscillatoria,* etc. Tindall with co-researchers described aerobic, alkaliphilic, extremely halophilic archaea belonging to genera *Natronobacterium* and *Natronococcus* (Tindall et al. 1984). *Methanobacterium thermalcaliphilum* and *Methanosalsus zhilinae* are examples of anaerobic archaea isolated from soda lakes. The aerobic heterotrophic spore-forming alkaliphilic eubacterium (belonging to the genus *Bacillus*) was isolated in 1983 from highly mineralized lake Wadi Natrun in Egypt. This bacterium was tolerant to salinity of more than 4 M NaCl (Weisser and Trüper 1985). Alkaliphilic anaerobic sugarlytic spirochetes also were found to be halotolerant, and some of them require NaCl for growth (Zhilina et al. 1996; Hoover et al. 2003). The discovery of alkaliphilic sulfate-reducing bacteria finally had confirmed the long-time existing hypothesis of Abd-el-Malek about functioning of sulfidogenes in hypersaline environments (Abd-el-Malek and Rizk 1963). After the application of specific media with

carbonate/bicarbonate buffers the presence of sulfate-reducers (*Desulfonatronovibrio hydrogenovorans, Desulfonatronum lacustre, D. thiodismutans,* and *D. cooperativum*) was shown in soda lakes of African, Asian, and American continents (Zhilina et al. 1997; Pikuta et al. 1998; Pikuta et al. 2003c; Zhilina et al. 2005). One of the alkaliphilic sulfate-reducers was found positioned very distantly from known phylogenetic lineages and represented the separate family *Desulfonatronaceae* (Kuever et al. 2005).[1]

The alkaliphilic enzymes from some alkaliphiles have optimal activity at pH much higher than physiological pH growth optimum of organisms, and this capacity is used quite well in biotechnology (detergents and wash powders).

Alkaliphilic microorganisms develop at high concentrations of Na^+ ions and, similarly to halophiles, have to accumulate osmoprotective compounds to resist the osmotic stress. Due to a high environmental pH the alkaliphiles must maintain their intracellular pH and realize different pathways for ion transport. All these properties of alkaliphilic organisms suggest that their adaptation to extreme environmental conditions during evolution had occurred at the system level (Zavarzin et al. 1999). According to academician Zavarzin, the basic difference between the alkaliphilic and halophilic microbial communities consists of the fact that soda mineralization resulted from the formation of athalassic inland water bodies rather than thalassic ones. For this reason, the prokaryotic alkaliphilic community can be regarded as a relic analogue of the continental biota in the early Proterozoic. In order to answer the question of whether the alkaliphilic community can indeed reflect the terrestrial source that gave rise to the biological diversity of prokaryotes, one should find out if the biodiversity of this community is sufficient to include representatives of all major branches of the universal phylogenetic tree of prokaryotes and show that the alkaliphilic community is not a dead-end line of evolution, like hyperthermophiles, out of which it is impossible to derive total diversity.

A recent review compared the mechanisms of pH homeostasis in neutrophilic bacteria and alkaliphilic bacilli (Padan et al. 2005). It was shown that active alkaline pH homeostasis that is important for both survival and growth, significantly depends upon monovalent cation/proton antiporters, the identification of which is generally achieved by combining genetic and biochemical approaches.

7. Microorganisms Resistant to Radiation

The beginning of a study of microorganisms resistant to high radiation was initiated after the remarkable discovery of *Deinococcus radiodurans* in 1956 during the process of food sterilization by X-ray radiation. However, in contrast to other types of extremophiles, there are no known microorganisms that are true radiophiles among the radio-resistant microorganisms. It is indeed difficult to prove that such types exist, since true radiophiles theoretically should not be able to live below certain fluxes of radiation, but should always require high irradiation levels for growth. Life without a certain level of radiation is impossible, and this feature of life unfortunately was never mentioned in any biological definition of life. The evolution of organisms that are able to grow continuously at 6 kilorads (60 Gy) h^{-1} or survive acute irradiation doses of 1,500 kilorads is remarkable, given the apparent absence of highly radioactive habitats on Earth over geologic times. Notwithstanding a few natural fission reactors like those that gave rise to the Oklo uranium deposits (Gabon) two billion years ago, the radiation levels in the Earth's surface environments, including its waters containing dissolved radionuclides, have provided only about 0.05 to 20 rads/year over the last four billions years (Makarova et al. 2001). DNA damage is readily inflicted on organisms by a variety of common physicochemical agents (UV light or oxidizing agents) or non-static environments (cycles of dessication and hydration or cycles of high and low temperatures) and it seems more likely that radiation resistance evolved in response to chronic exposure to non-radioactive forms of DNA damage. In the exponential growth phase, cells of *D. radiodurans* do not die in response to ionizing irradiation up to 0.5 megarad and they show 10% survival at 0.8 megarad (Moseley and Evans 1983). The resistance to UV for *D. radiodurans* cells is 33-fold more than for *E. coli* cells. After complete study of *D. radiodurans* R1 genome (Makarova et al. 2001) the following conclusions were made: (1) The expanded Nudix hydrolase superfamily and the homologs of plant desiccation resistance-associated proteins are likely to contribute to both the extreme radiation and the desiccation resistance of *Deinococcus*; (2) A variety of other proteins, particularly those that belong to expanded families, are likely to be involved in the unusual phenotype of this bacterium; (3) The unexpectedly numerous nucleotide repeats may play a role in stress response; (4) The transferred entire gene systems, such as genes encoding pili-associated functions, could contribute to the formation of surface structures involved in secretory systems similar to the type III secretion pathway (since there is no experimental data concerning pili formation in *D. radiodurans* cells). The conclusion after other proteomic research of *D. radiodurans* (Mrázek 2002) suggests the application of this organism for bioremediation of radioactive waste sites. Earlier Daly showed a realistic approach for engineering *D. radiodurans* for bioremediation, metal remediation, and toxic organic compound degradation (Daly 2000). The latest comparative genomic research of *D. radiodurans* and *Thermus thermophilus* was performed by Omelchenko et al. (2005).

Other radiation resistant bacteria were found during food sterilization (Hastings et al. 1986; Lacroix and Lafortune 2004). Seven strains of *Lactobacillus sakei* were more resistant to gamma-irradiation in the exponential (log) phase (0.82 kGy) than in the stationary phase of growth (0.64 kGy). *Pantoea agglomerans* was found to be a natural contaminant of carrots

[1]The original spelling, *Desulfonatronumaceae* (sic), has been corrected on validation according to Rule 61. Reference: Lists editor, *IJSEM*: Validation list n° 107 (footnote). *Int. J. Syst. Evol. Microbiol.,* 2006, 56: 1–6.

and radiation resistant bacterium (it survived after 0.15 kGy doses).

Much research dealing with the isolation of radiation-resistant bacteria without exposure to irradiation were performed. Some strains were selected by the resistance to desiccation, and others by sampling hot spring habitats (Sanders and Maxcy 1979; Albuquerque et al. 2005). A radiation resistant moderately thermophilic species *Truepera radiovictrix* was described during the study of isolates from hot spring runoffs on the Island of São Miguel in the Azores; this species phylogenetically was very distant from known genera and families, and exhibited 60% survival after exposure to 5.0 kGy of gamma irradiation.

Anellis and his colleagues performed experimental measurements on the survival of different strains during Cobalt gamma irradiation at different temperature regimes (Anellis et al. 1973). It was shown that at low temperatures (–80°C) during ionization the most resistant microorganisms were: *D. radiodurans, Streptococcus faecalis,* and *Alcaligenes faecalis*.

Experiments with the desiccation-tolerant cyanobacterium *Chroococcidiopsis* demonstrated high resistance of this microorganism to 15 kGy X rays irradiation (Billi et al. 2000). The authors hypothesized that the capacity of *Chroococcidiopsis* cells to repair radiation-induced DNA damage and probably also desiccation-induced DNA damage is related to the ability to use redundant genetic information, as *D. radiodurans* does.

Other investigations have established that a vacuum of 10^{-6} Pa decreased the survival of desiccation-resistant microorganisms, and that exposure to space vacuum caused a greater decline in survival when compared to ground vacuum. The exposure to space vacuum ($\sim 10^{-6}$ Pa) decreased cell survival by two and four orders of magnitude for *Bacillus* sp. PD3D and *D. radiodurans* respectively (Saffary et al. 2002).

Another interesting experimental work was performed with measurement of metals inside cells of radio-resistant bacteria (Ghosal et al. 2005). Researchers had shown that radioresistant bacteria accumulate exceptionally high intracellular manganese and low iron levels. The opposite effect was found for dissimilatory metal-reducing *Shewanella oneidensis* that accumulates Fe but not Mn, and is extremely sensitive to radiation. The authors proposed that for Fe-rich/Mn-poor cells, death by radiation is caused by released Fe(II) from proteins, that leads to additional cellular damage by Fe(II)-dependent oxidative stress. In contrast, Mn(II) ions accumulated in cells of *D. radiodurans* might have the function of antioxidants that reinforce enzymic systems, which defend against oxidative stress during recovery. The conclusion was: It is necessary to include considerations of respiration, tricarboxylic acid cycle activity, peptide transport and metal reduction as potential targets to control recovery after radiation injury.

The effect of UV-B radiation on aquatic ecosystems was comprehensively discussed in Häder's review (Häder 2000). It was concluded that the consequences of significantly enhanced levels of exposure to UV-B radiation include losses of biomass, such as food sources for humans, changes in species composition, decrease in availability of nitrogen compounds and reduced sink capacity for atmospheric carbon dioxide, resulting in the potential augmentation of global warming. According to the author, there is significant evidence that increased UV-B exposure is harmful to aquatic organisms, however damage to ecosystems is still uncertain. Responses to increased solar radiation will not be limited to simple decreases in primary production. In fact, shifts in community structure may be more common and result in little detectable differences in ecosystem biomass.

The number of species in the genus *Deinococcus* was recently increased by an additional 9 species isolated from the Sonoran Desert in Arizona (Rainey et al. 2005), and currently this genus includes 20 species. The genera *Acinetobacter, Hymenobacter, Kineococcus, Kocuria, Methylobacterium* (eubacteria), and *Thermococcus, Pyrococcus* (euryarchaeotae) also include species and strains that are resistant to ionizing radiation.

8. Extremophiles in Soils

The diversity of extremophilic organisms in soils is quite high, exceeding our knowledge and what we once expected. This field is just beginning to be explored and appreciated. With the advancement in molecular techniques and the increasing availability of environmental rDNA sequences, future research can be done to look into the community structure as well as the microbial processes in which these microbes are engaged.

Microorganisms were known as the most abundant species in soil for some time, and their diversity was enormous. The paper published by Torsvik et al. (1990) indicated there were 4,600 distinct prokaryotic genomes per gram of soil based on DNA reassociation kinetics. A decade later, they surveyed different types of soil and estimated 10^{10} cell/cm^3 with up to 8,800 genome equivalents in pasture soil (Torsvik 2002). The effort to study the composition and distribution, however, has only met with limited success. This is mainly due to the difficulties in cultivating and characterizing soil microbes by traditional, culture-based methods. As a result, cultivable species only represented less than 0.1% of the diversity (Bintrim et al. 1997).

In recent years significant progress has been made to assess microbial communities based on a variety of culture-independent methods and techniques that were developed. A number of studies utilized ribosomal RNA operon, typically the small subunits such as16S and sometimes 5S rRNA, to analyze the population in soils (Stackebrandet 1993; Ueda 1995; Hofle 1999; Borneman 1997; Zhou 1997). Environmental sequencing projects targeting 16S rRNA also allowed a look at the diversity of uncultured organisms (DeLong and Pace 2001; Tringe et al. 2005). Several techniques based on PCR amplification of DNA extracted from soil samples also became available. These include ribosomal intergenic spacer analysis (RISA), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single-strand conformation polymorphism (SSCP), random amplified polymorphic DNA (RAPD),

and amplified ribosomal DNA restriction analysis (ARDRA). The fingerprints generated from these methods enable the investigation of community compositions as well as study the differences due to treatment or biogeographic changes (Kent 2002; Fierer 2006).

The PCR-based methods are generally easy to perform and they have made significant contribution in soil community analysis. In spite of their popularity and frequent usage, these methods are not without limitations. The procedures often introduce variables, which result in biases in detecting certain populations. Some limitations include: cell lysing methods possibly affect DNA recovery from certain bacteria; extracting DNA from soil samples not always capturing microbes in low numbers; and various inhibitors in soil impacting the amplifying capability of PCR.

In order to circumvent and minimize biases, different approaches have developed in recent years to improve detection and resolve amplified DNA fragments. These include length heterogeneity PCR (LH-PCR) (Ritchie et al. 2000) and terminal restriction fragment length polymorphism (T-RFLP) (Kitts 2001; Marsh et al. 2000; Osborne et al. 2000). Alternatives to PCR methods have also been used; examples include Fatty Acid Methyl Ester (FAME) profiles of cell membrane (Ibekwe and Kennedy 1998) and fluorescence in situ hybridization (FISH) to perform direct microscopic examination (Amann et al. 2001). The latter technique also allows specific groups of interest to be targeted by customizing the fluorescent probes for hybridization (Amann 1995; Raaijmakers and Weller 1998).

Microbial Diversity in Soils

Many environmental factors influence prokaryotic diversity in soil. These include: soil moisture, particle size, pH, organic carbon concentration, carbon to nitrogen ratio, and vegetation type. Fierer and Jackson's study (2006) indicated bacterial diversity was unrelated to site temperature, latitude, and other variables that typically affect plant and animal diversity, and the composition of a community was not dependent on geographic distances. Instead, the pH of soil was the best predictor of bacterial diversity and richness, as well as community composition. Bacterial diversity was highest in neutral soil, and the level of diversity and richness decrease as pH moves towards acidic conditions. The authors did not know whether this phenomenon extended to the other extreme, because basic soil (pH > 8.5) samples were not easy to obtain, so it is unclear if diversity plateaus in soils with neutral pH.

Microbial diversity is influenced by the particle size of soil (Ranjard et al. 2000). One study indicated α-Proteobacteria dominated in large particle soil, whereas *Halophaga*/*Acidobacterium* were the most common organisms found in clay particles (Sessitch et al. 2001).

There were many studies showing bacterial diversity, or community composition, from different parts of the world (Liles et al. 2003; Nemergut et al. 2005; Wery et al. 2003; Nagy et al. 2005). Liles et al. (2003) analyzed the diversity of microbial genomes by constructing a metagenomic DNA library from soil samples collected in Madison, Wisconsin. The rDNA genes indicated the majority of the bacteria belonged to the Proteobacteria, Acidobacteria, Cytophaga-Flexibacter-Bacteroides (CFB), Firmicutes and Verrucomicrobia groups (76–86% within a three-year span). The predominant population belonged to members in Acidobacteria. Within the Proteobacteria, all subdivisions of alpha, beta, gamma and delta-Proteobacteria were present. Their proportions varied depending on the molecular approaches being used. The bacterial sequences obtained from a sandy ecosystem near Darmstadt, Germany showed similar diversity as to the phyla and groups being detected (Ochsenreiter et al. 2003).

Acidobacteria was the predominant group in the Colorado Rocky Mountains alpine tundra soil (Nemergut et al. 2005). They were most abundant during spring, summer and winter months; and they also presented in high numbers in both wet and dry meadow samples during springtime. Another study which surveyed the prokaryotic diversity in Arizona Sonoran Desert soil crusts showed Acidobacteria-like clones ranked highest in arid soils, and their DNA was among one of the major groups being amplified and identified. On the other hand, these bacteria were low representatives in agriculture soils (Nagy et al. 2005). These findings were consistent with those of Dunbar et al. (1999) where *Acidobacterium*-related organisms comprised nearly half of the identifiable bacteria in their clone libraries from four arid soils in Northern Arizona. Furthermore, a wheat field from Holland also yielded similar results, showing *Acidobacterium* was one of the dominant groups of uncultured organisms (Smit et al. 2001).

Acidobacteria are considered extremophiles, capable of growing under unusual conditions when compared with other soil inhabitants such as Proteobacteria, Actinobacteria, and Firmicutes. The type species, *Acidobacteriuim capsulatum*, was an acidophilic chemoorganotroph isolated from acidic mineral environment (Kishimoto et al. 1991). Since its name was validated in 1991, members of this genus have been found in a variety of soils as mentioned above, and not only limited to acidic habitats.

Other extremophilic organisms existed in soil, especially if the environmental conditions of the soil are considered extreme; i.e. Antarctic soil, alkalinic sediments, acidic forest cambisol (Wery et al. 2003; Dunfield et al. 2003; Wiegel and Kevbrin, 2004). Many of these extremophiles belong to the Bacteria Domain. It is not surprising to find a number of psychrotrophs in Antarctic soils; for example, species of *Psychrobacter* and *Planococcus* as well as other ubiquitous genera (i.e., *Chryseobacterium, Brevundimonas, Paenibacillus*) that could tolerate low temperatures (Wery et al. 2003). Then there are halophilic organisms residing in saline soils; *Marinococcus halotolerans* (Li et al. 2005), *Streptomonospora alba* (Li et al. 2003), and *Alkalibacterium iburiense* (Nakajima et al. 2005) just to name a few from recently published names. Thermophilic organisms continued to be discovered in soils with higher temperature; for example,*Anaerobranca gottschalkii* is a recently described thermoalkaliphile isolated from a humid soil of a hot

lake inlet in Kenya (Prowe and Antranikian 2001), and *Geobacillus tepidamonas* originated from a geothermally heated soil in Yellowstone National Park (Schäffer et al. 2004). Examples in acidic niches are *Methylocella silvestris*, a methanotroph from an acidic forest cambisol (Dunfield et al. 2003), and two Clostridia *Cl. akagii* and *Cl. acidisoli* from acidic peat-bog soil (Kuhner et al. 2000).

Then there existed some extremophiles in unexpected places. Echigo et al. (2005) reported halophilic bacteria inhabiting nonsaline environments in areas around Tokyo, and most of them belong to the *Bacillus* and related genera. A couple of thermophilic spore formers were isolated from the cool soil environment in Northern Ireland (Banat et al. 2004). Alkaliphiles have been isolated from a variety of mesobiotic and neutral soils and sediments (Wiegel and Kevbrin 2004). Several studies have found sequences from mesophilic soil habitats worldwide, and they belong to the Crenarchaeota phylum in the Archaea Domain by phylogenetic association (Sliwinski and Goodman 2004; Simon et al. 2005; Ueda 1995).

Archaea in Soils

Crenarchaeota. The presence and significance of Archaea in soils were not recognized until a decade ago. This was mainly due to the fact that Archaeal members were mostly isolated from extreme environments or unique ecological niches, and they could not be easily cultivated and studied by traditional microbiological methods. In 1997, Bintrim and colleagues demonstrated the presence of an archaeal population in soil, and they were more widespread than once realized (Bintrim et al. 1997). By amplifying and cloning the small subunit ribosomal RNA genes of Archaea from soil samples collected in Madison, Wisconsin, a cluster of these organisms was found to represent a component of the microbial communities in these soils. Phylogenetic analysis of these clone sequences indicated this lineage was within the Crenarchaeota phylum, but its branch was distant to any cultivated member of the Archaea. In fact, it is affiliated with the Group I of the marine Archaea, which is composed of members in the planktonic division of the Crenarchaeota.

Subsequent studies confirmed this unexpected finding, that nonthermophilic members of Crenarchaeota were ubiquitous and abundant in soil. This particular lineage of Crenarchaeota was consistently present in the different soils sampled from diverse geographic areas in Europe and Asia, as well as from two microbial mats (Ochsenreiter et al. 2003). A survey from soil samples in Darmstadt, Germany revealed the relative abundance of crenarchaeotal rDNA was between 0.5–3% in bulk soil. In the rhizosphere of a sandy ecosystem and an agricultural setting, these archaeal sequences represented 0.16 and 0.17% of the populations respectively. Thus, soil crenarchaeota constitute a stable and unique component of the inhabitants in terrestrial environments (Ochsenreiter et al. 2003).

Furthermore, these crenarchaeota were found in association with plants. Their small subunit rRNA gene sequences were present in rice rhizosphere (Großkopf et al. 1998b), plant roots such as maize and tomato (Chelius and Triplett 2001; Simon et al. 2005), and mycorrhizospheres of pine seeding (Bomberg et al. 2003). Using epifluorescence microscope and culture-independent recovery of small subunit rRNA gene sequences, Simon et al. (2000) showed soil Crenarchaeota colonized both young and senescent plant roots at an unexpected high frequency. In fact, they were particularly good colonizers of senescent roots, thus, these organisms had important implications for root development and ecology.

Sliwinski and Goodman used PCR-single stranded conformation polymorphism (PCR-SSCP) to compare the crenarchaeal consortia inhabiting the rhizosphere with those present in bulk soil at different locations in Wisconsin. They showed the profiles from rhizosphere were distinct from those obtained in corresponding bulk soil. This difference seemed to result from the increased richness in rhizosphere as compared to bulk soil, and it was not dependent on plant lineage. Therefore, the authors concluded that the crenarchaeal associations with plants in native environments reflected the interactions between plant and microbes, and their relationship was mediated by environmental factors (Sliwinski and Goodman 2004).

Archaea also reside in upland pastures as shown by Nicol et al. (2004). Managed pastures with inorganic nitrogen fertilizer input had a different crenarchaeal community than those from unmanaged upland pastures (i.e., native grassland) where their nitrogen sources were largely from grazing sheep. The archaeal communities were very stable in these soil microcosms, and they were not affected by nitrogen sources amendments and pH increases.

All these results indicate mesophilic soil crenarchaeotes play an important ecological role (Bintrim et al. 1997) and their association with plant roots is significant. Until recently, all the studies were done by culture-independent methods and no member has been isolated and identified. Some progress began to surface lately, as the first evidence of culturing mesophilic crenarchaeotes was reported. These organisms responded positively to root extract enrichment although the actual substrates supporting their growth were still unknown (Simon et al. 2005). As novel cultivation methods continue to be developed, the axenic culture of crenarchaeotes will hopefully appear in the near future.

Euryarchaeota. Other archaeal diversity has been found in different types of soil in addition to Crenarchaeota. By using 16S rRNA PCR amplification, Utsumi et al. (2003) surveyed peat soil samples from geographically distinct wetlands, ranging from the cold West Siberia bogs to the subtropical Okefenokee swamp in the US. Most of the sequences were related to the methanogens in the Euryarchaeota phylum. This finding was not surprising, since methanogens were among the earliest members recognized in the Archaea Domain. Many genera and species have been described since, in spite of their obligate anaerobic characteristics (Euzéby 2006).

Most of the archaeal community studies on Euryarchaeota were done in paddy field soil, especially from flooded rice paddies where these organisms exist as one of the major biogenic

sources of atmospheric methane. Methanogens are the most functionally important archaea in these environments due to the anoxic condition caused by flooding (Liesack et al. 2000). These organisms utilize H_2-CO_2 or acetate as substrates for methanogenesis, and they can survive during the dry and oxic periods. The majority of methanogens are found in the anoxic bulk soil of flooded rice microcosms; in fact, between 10^6 to 10^7 cells of H_2-CO_2 utilizing methanogens were detected per gram of dry soil in Italy (Großkopf et al. 1998a). Reports on Italian and Japanese rice paddy soils seem to indicate *Methansarcinaceae* (i.e., species *M. barkeri, M. mazei*) and Methanosaetaceae (species *M. concilii*) were the predominant acetate-utilizing methanogens; whereas members of the Methanobacteriaceae family (species *M. bryantii*, *M. formicicum*) were the major group found in hydrogenotrophic methanogens (Liesack et al. 2000; Großkopf et al. 1998a; Min et al. 1997). These diverse patterns of methanogenic organisms were influenced by environmental factors such as acetate concentration, temperature, and soil particles size (Liesack et al. 2000; Großkopf et al. 1998a; Chin et al. 1999; Ramakrishnan et al. 2000). In addition to the known methanogenic species, Liesack et al. (2000) noticed several novel archaeal lineages that have been detected in the anoxic bulk soil, rice roots of flooded rice microcosms, and in slurries of Italian rice paddy soil. Pure cultures were not available from these lineages and only a phylogenetic branching pattern was noted. One of the rice clusters grouped on the same branch as *Thermoplasma acidophilum* and marine group II, but it only had a distant relationship. The other rice cluster formed a novel main line of decent without any affiliation to any other euryarchaeal groups (Großkopf et al. 1998b). Obviously there are members in the Euryarchaeota phylum waiting to be discovered in order to study and elucidate their ecological roles.

9. Astrobiological Significance of Extremophilic Microorganisms

The study of metabolically active microorganisms at low and/or subzero temperatures is of major interest to astrobiology, since most bodies of our Solar System are frozen worlds.

It has also been shown that microorganisms can remain viable for geological periods of time when cryopreserved in ice (Abyzov et al. 2004; Pikuta et al. 2005a). It is well known that microbes are generally capable of remaining viable for very long periods of time when freeze-dried (lyophilization) and that some organisms can maintain metabolic activity at temperatures as low as –20°C. Representatives of the genus *Trichococcus* have been found to be capable of replication and growth at –5°C at both aerobic and anaerobic conditions (Pikuta et al. 2006a).

The microflora of the cryosphere of planet Earth provides the best analogs for life forms that might be found in the permafrost or polar ice caps of Mars, near the surface of the nuclei of comets, or in the liquid water and the ice crusts of icy moons of Jupiter and Saturn (Hoover et al. 2002; Hoover and Pikuta 2004; Abyzov et al. 2006). It is now well established that water ice exists today in a vast deposit at the North Polar Ice Cap of Mars (Zuber et al. 1998; Bass and Paige 2000; Bass et al. 2000; Kieffer et al. 1976; Greve 2000; Haberle and Jakosky 1990). The Mars Global Surveyor had produced images of double-rimmed polygons on Mars that are consistent with the double-rimmed polygons in permafrost of Earth that are produced by expansion and contraction effects associated with the seasonal water-ice freeze-thaw cycles (Paepe et al. 2001). The High Energy Neutron detector aboard the Mars Odyssey spacecraft (Litvak et al. 2006) has also found that there are large water-rich permafrost areas, with contents up to 50% water by mass fraction, at both the North and South polar regions of Mars. In the northern latitudes, these water-ice layers of permafrost are very near the surface, but in the south they are covered by a thicker (10–20 cm) layer of soil. The water content of the Mars regolith was found to exceed 11% at latitudes greater than 60 degrees in both hemispheres. Two regions near the equator (Arabia Terra and Medusae Fossae) even exhibited 9–10% water (Mitrofanov et al. 2004) with the lower layer of soil in the moistest region (30 E, 10 N) having soil with over 16% of water by mass which is comparable to the water content (13–19%) of tropical rain forest soil on Earth (Odokuma and Dickson 2003). The daytime atmospheric temperatures on the surface of Mars were measured by Pathfinder to sometimes exceed 20°C that could yield diurnal films of liquid water due to melting of near surface ice between the interstices of permafrost grains. This could provide conditions suitable for current life on Mars (Levin and Levin 1998).

The possibility that microbial extremophiles might thrive today (or be present in a cryopreserved state) in the water ice and permafrost in the modern Martian regolith is of great significance to Astrobiology. While it is generally accepted that the one common bond for all life known on Earth is the universal requirement for water, it is clearly not necessary that the water be found in liquid state in large bodies, such as seas or lakes. Many microorganisms grow within thin films of water in permafrost and in frozen rocks of the polar regions (cryptoendoliths). Microorganisms also inhabit acidic (acidophiles) and brine channels (halophiles) in glaciers (Hoover et al. 2002; Pikuta and Hoover 2004b; Hoover and Gilichinsky 2001; Mancinelli et al. 2004). On Earth, solar heating of low albedo rocks entrained in glaciers results in microenvironments (cryoconite ecosystems) with trapped liquid water, gasses, organic chemicals, and minerals that provide ideal conditions for the growth of microbial communities that include both prokaryotic and eukaryotic microorganisms. The study of the species of Earth's microflora that thrive in the coldest regions of our planet is of great importance to the design and development of robotic systems that will be needed to search for biomarkers and evidence of past or present life on Mars, Europa, and other prime targets for Astrobiology research.

The importance of studying acidophilic microorganisms is primarily concerned with acidic thermal systems such as may exist on bodies of our solar system that have volcanic or tectonic activities. Evidence for ancient volcanic activity on Mars and Venus, and recent volcanic activity on Io has been revealed by the *Viking, Pathfinder, Global Surveyor, Mars Odyssey,*

Voyager, Venera, and *Magellan* space missions with optical, ultraviolet, infrared, and radar images and IR, gamma-ray, and neutron spectral data (Pikuta and Hoover 2004b). Much volcanic activity has also occurred on Venus, which is known as the most acidic planet of the Solar System. Venus is thought of as a twin planet of Earth although it rotates in the opposite direction. However it is much closer to the Sun and it has a surface temperature of around 470°C, and an atmospheric pressure that is equivalent to 100 times that of Earth's atmosphere. After the discovery (Harris et al. 2002) of active microorganisms in Earth's atmosphere at an altitude of 44 km (level of stratosphere) the point of view about possible existence of life forms on acidic droplets in the cooler dense clouds of the Venus atmosphere has been discussed.

The Jovian moon Io presents the most active volcanic body of our Solar System with conditions that may be acceptable for the development of sulfur-dependent microorganisms. Io's volcanic activity could also provide acidic geysers and springs suitable for the development of acidophilic sulfur-metabolizing lithotrophs. There is also much evidence that beneath the fractured ice crust of Europa, the liquid water ocean exists. The Jovian tidal effects and/or hydrothermal vents could provide the heat necessary for the liquid water ocean underneath the crust of this icy moon. It is conceivable that acidophilic microorganisms might also inhabit acidic hydrothermal vents on Europa (Pikuta and Hoover 2004a), and that psychrophiles could thrive in niches at interfaces between the liquid water and the ice sheet.

The importance of the study of alkaliphilic microorganisms for Astrobiology was enhanced by the findings of abundant carbonates and carbonate globules rimmed with possibly biogenic magnetites in association with the putative microfossils in the ALH84001 meteorite (McKay et al. 1996; Wadhwa and Lugmair 1996). The study of this meteorite provided definitive evidence of the existence of carbonate minerals on Mars. The participation of microbial agents in formation of carbonate systems on Earth was discussed previously (Pikuta et al. 2003a). Even though the ALH84001 nano-structures were not of sufficient size as to be certainly accepted as valid recognized microfossils, these results profoundly stimulated the study of meteorites, bacterial paleontology, and microbial extremophiles. The extensive research efforts triggered by the ALH84001 results have helped to define valid biomarkers and to delineate where and how to best search for evidence of life in Earth's most hostile environments and elsewhere in the Solar System.

Recent studies have resulted in the detection of evidence for the mineralized remains of a large and complex assemblage of diverse coccoidal and filamentous microorganisms and cyanobacterial mats in the Orgueil (CI1) and Murchison (CM2) carbonaceous meteorites (Hoover 2005; Hoover 2006). These forms are much larger and more complex than the ALH84001 nanostructures and they exhibit features that provide clear associations with known morphotypes of all five orders of the Cyanobacteriaceae. Some of the forms occur as spherical and irregular coccoids, sometimes in colonies with electron transparent carbonaceous mucilaginous envelopes and are interpreted as morphotypes with characteristics of the Chroococcales. Others are in pseudo-filaments with baeocyte-like forms consistent with the Pleurocapsales. These morphologically simple forms are not so distinctive as the larger forms that occur as uniseriate and multiseriate unbranched and branched filaments and mats that have been found embedded in the meteorite rock matrix. The vast majority of the filamentous forms found in freshly fractured surfaces of the meteorites are consistent in both size and detailed morphological features with the carbon-rich sheaths, and mineralized trichomes of filaments of the Oscillatorianlean cyanobacteria. However, the Orgueil meteorite has also been found to contain a number of other forms that have tapered polarized filaments and exhibit cross-wall constrictions, true and false branching and highly differentiated cells. Many of the tapered filaments appear to represent benthic forms attached to the substratum by basal heterocysts (occasionally exhibiting ellipsoidal akinetes) with detailed features characteristic of morphotypes of members of the genera *Calothrix*, *Rivularia*, and *Tolypothrix*. The most distinctive of the Orgueil filaments are exceptionally well-preserved forms that are interpreted as representing morphotypes of the Orders: Nostocales and Stigonomatales. These microfossils have extremely complex and distinctive biogenic characteristics that cannot be confused with minerals and other abiotic microstructures. Energy Dispersive X-ray Analysis has revealed that they have anomalous C/O, C/N, and C/S ratios that clearly establish that they cannot be attributed to recent biological contaminants and the forms have been interpreted as indigenous microfossils. The detection of microfossils (Hoover 2007) in carbonaceous meteorites supports the hypothesis proposed by Hoyle and Wickramasinghe in 1981 that comets and meteorites could be carriers and distributors of living cells in the Solar System (Hoyle and Wickramasinghe 1981; Hoover et al. 1986).

Microbial Extremophiles Investigated for Astrobiology Research

It is well known that microbial extremophiles are of great importance in the rapidly emerging field of Astrobiology in order to help us understand where and how to search for evidence of life elsewhere in the Cosmos. Research carried out in the NASA/NSSTC Astrobiology Laboratory has used a variety of samples collected during field expeditions to a number of the most extreme environments on Earth. These included:

1. Samples from cold regions of our planet—frozen Pleistocene thermokarst ponds, ice wedges, and permafrost of Fox Tunnel and Glaciers of Alaska; permafrost of the Kolyma lowlands of Northeastern Siberia; guano of the Magellanic penguins of southern Patagonia, Chile; and snow, rocks, and deep ice cores from the Patriot Hills, Thiel Mountains, and Vostok Station, Antarctica;
2. Samples from acidic ecosystems—Chena Hot Springs near Fairbanks, Alaska;
3. Samples from halo-alkaline ecosystems—-soda lakes in California;

4. Samples from deep-sea hydrothermal vents—Rainbow Deep Sea Vent, Azores, Middle Atlantic;
5. Samples from geysers and moderate thermal springs—Alaska and California.

The following groups of microorganisms were isolated and studied. Five strains of alklaliphilic mesophilic bacteria were isolated in pure cultures and described from halo-alkaline systems of Owens Lake and Mono Lake in California. Four of them have been validly published and deposited in International Microbial Culture Collections:

1. *Spirochaeta americana* ASpG1^T was found to be the first obligate anaerobic and alkaliphilic free-living sugarlytic spirochete on the American continent; the peculiar feature of this unique strain is production of molecular hydrogen as a major end metabolic product that offers potential for biotechnology applications as an alternative fuel for the strategy of the Hydrogen economy;
2. *Desulfonatronum thiodismutans* FML1^T was also found to be the first obligately alkaliphilic sulfate-reducing bacterial species on the American continent, and as a specific feature of this strain differentiating it from Asian strains is that it has a chemolithotrophic metabolism that allows this bacterium to grow exclusively on hydrogen and CO_2 without any organic molecules;
3. *Tindallia californiensis* APO^T was described as an agent responsible for decomposition of proteolysis products and some organic acids (Pikuta et al. 2003d); This species performs the function of a secondary anaerobe in anaerobic alkaliphilic microbial community of Mono Lake; This strain is capable of respiration by performing a Stickland reaction on certain pairs of amino acids;
4. *Anaerovirgula multivorans* SCA^T was found as the cellulolytic agent in the anaerobic microbial community of Owens Lake, but sequent study showed this process as very weak. The major trophic function for this species is degradation of proteolysis products molecules and some organic acids. This strain was found to be phylogenetically distant from known clostridial species and had taken a separate lineage on the separate genus level and separate species (Pikuta et al. 2006b);
5. *Spirochaeta* sp. ASpC2^T is now in preparation for publication; this obligately anaerobic and alkaliphilic strain was isolated from a cellulolytic enrichment culture from Owens Lake, and it has a number of physiological features differentiating it from other known free-living spirochetes.

From the acidic system of Chena Hot Spring in Alaska the obligately acidophilic strain AGC2^T was isolated in pure culture (Pikuta and Hoover 2004a). This mesophilic, spore-forming strain is able to grow on the medium with high concentrations of Fe^{2+} with pH 1.5–2.0. The taxonomic description of this strain is currently in preparation.

From a deep-sea hydrothermal vent the obligately sulfur-reducing archaebacterial strain OGL-20P^T was isolated and described. This isolate belongs to a separate new species of the genus *Thermococcus* with proposed name *T. thioreducens* (Pikuta et al. 2007).

The study of samples from cold regions led to discovery of psychrotolerant *Carnobacterium pleistocenium* FTR1^T that was alive after remaining frozen for 32,000 years in the Pleistocene ice of a Thermokarst pond of the CRREL Fox Permafrost Tunnel near Fairbanks, Alaska. This facultatively anaerobic fermenting bacterium was described as a separate new species since it was different from known species both on genetic and phenotypic levels (Pikuta et al. 2005a). This bacterium is of great importance to both Astrobiology and Paleontology. It is first a validly published species of the Pleistocene period and it was cryopreserved in ancient permafrost. It is also important to cryobiology by demonstrating that it is possible to revive living microorganisms from the ice and permafrost samples of other bodies of the Solar System.

Another interesting psychrotolerant sugarlytic bacterium *Trichococcus patagoniensis* PmagG1^T was isolated from penguin guano, and it was able to grow at -5°C at aerobic and anaerobic conditions. This record is the lowest known temperature for growth of a pure culture (Pikuta et al. 2006a). The description of the proteolytic psychrotolerant bacterium strain PPP2^T that represents a separate new genus and species is also in preparation (source of isolation is also guano from the Magellanic penguin).

INTERPRETATION AND CONCLUSION

If we consider the distribution of life in a matrix of pH/temperature, salinity/temperature, salinity/pH, radiation/temperature by placing the names of described known species of microorganisms on the corresponding matrix, the picture will be as follows. The diagram pH/temperature (Figure 1a) shows a gray painted square in an area of neutral pH and mesophiles/moderate-thermophiles limits; this square represents the region of a majority of known described microbial species that includes saprophytes, pathogens, and environmentally important agents responsible for the balance of trophic chains in surrounding ecosystems. On the left side of this square the distribution of acidophilic species is shown. As we can see, no acidophilic psychrophilic species have been found and described; that provides evidence for our point of view concerning the primary origin of acidophiles on a hot, cooling Earth. The area of hyperthermophilic and moderately thermophilic species of acidophiles is quite busy and is most extensively "populated" by a species of archaea. The most thermostable species is *Pyrolobus fumarii* that could survive 30–60 min in an autoclave at 120°C, and the neutrophilic but not validly published strain 121 that grows at a temperature higher than 120°C and survives for a short period of time at 130°C. The most alkalitolerant described species of archaea among the hyperthermophiles is *Thermococcus alkaliphilus,* which is able to grow at pH 10.5. On the

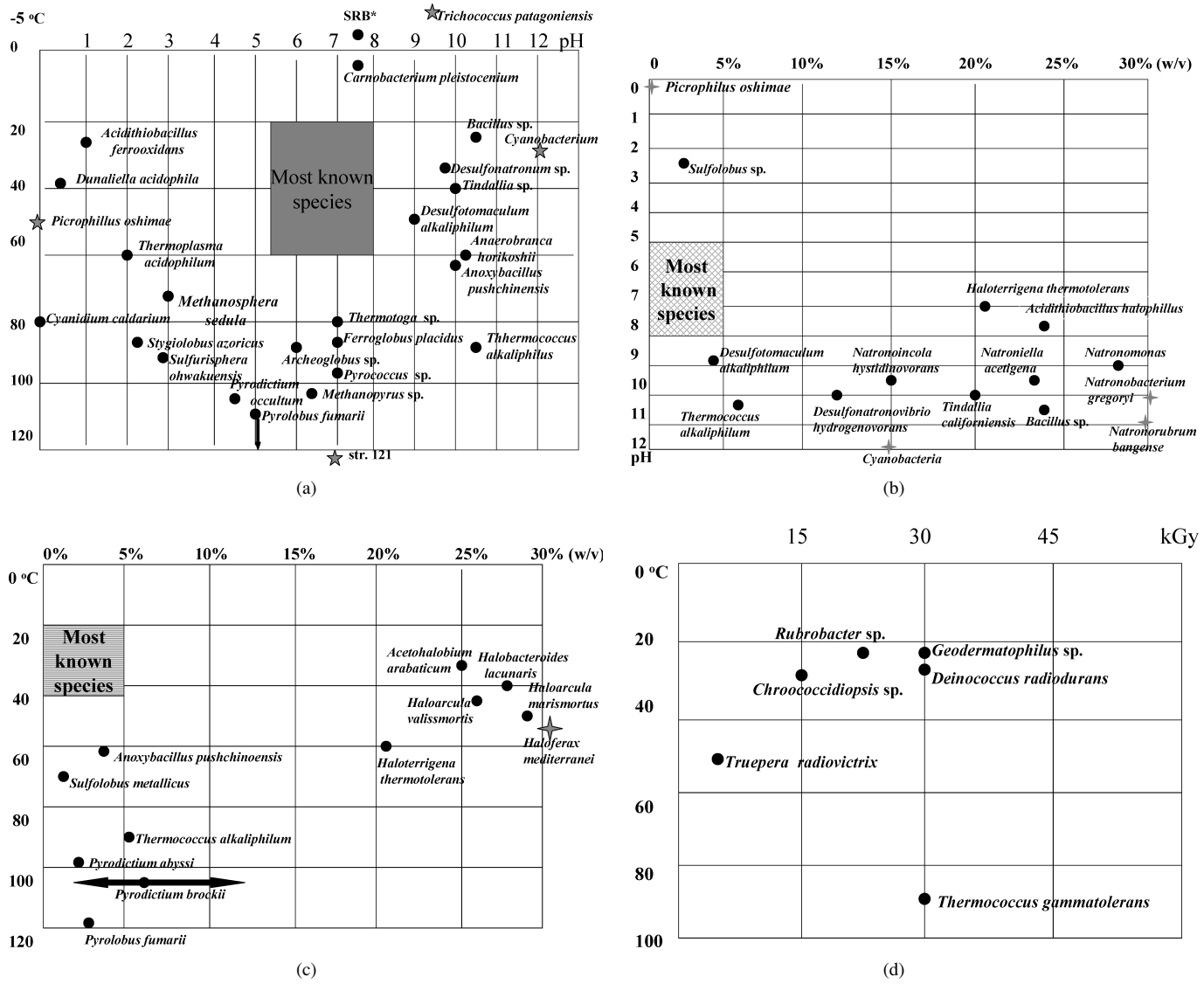

FIG. 1. Distribution of known species of microorganisms in the matrix: (a) pH/Temperature, °C; * SRB- 5 genera of psychrophilic sulfate-reducing bacteria (Knoblauch et al. 1999); (b) Salinity, % (w/v)/pH; (c) Salinity, % (w/v)/Temperature, °C; (d) Radiation, kGy/Temperature, °C.

right side of the gray square the distribution of mesophilic and moderately thermophilic species of microorganisms is shown; it includes bacteria, cyanobacteria, and archae-bacteria. As was discussed previously, mesophilic cyanobacteria are the leaders in alkalitolerancy among all living organisms. The recently described psychrotolerant species *Trichococcus patagoniensis* is able to grow at pH 10.0 and its other specific feature is the ability to grow at -5°C. Before the discovery of this species the record of growth at subzero temperatures was established for psychrophilic species of sulfate-reducing bacteria.

The diagram of salinity/pH (Figure 1b) shows the distribution of known species of microorganisms within this matrix. On the diagram on the left side the gray painted square represents the area of a majority of known species of microorganisms (saprophytes, pathogens, and environmental agents). As we can see, the extreme acidophiles appear in regions of salinity before 5%. Species of the genera *Natronobacterium* and *Natronorubrum* are able to grow at 30% (w/v) salinity. Cyanobacteria are spread to the pH 12 area and limited by 15% salinity. This diagram, once again, confirms the hypothesis about the later appearance of halophily during the evolution of life. The accumulation and solubility of Na^+ and Cl^- ions in water became possible only in the latest stages of Earth's evolution.

The diagram of salinity/temperature (Figure 1c) shows the presence of a mesophilic and moderately thermophilic species of microorganisms in an extreme saline area (25–30%); None of the hyperthermophilic species are known at extreme salinity. Probably, again, the rule of evolution on Earth is working: the first organisms were non-halophilic hyperthermophiles; the primordial ocean was not salty at all.

The final diagram (Figure 1d) shows the distribution of known species in the matrix of radiation/temperature. Three species (one of them an archaebacterium) could tolerate 30 kGy irradiation level. Does that suggest that the level of irradiation on Early Earth was around this number? Or is it possible that these microorganisms originated elsewhere in the cosmos where radiation levels were very high and were transported by comets and meteorites to the Early Earth? In order to resolve these very important questions there is a great need for Science to have more data since this area of extremophily has not been very thoroughly investigated.

Looking at these diagrams the logical question raises: What direction of biological changes (adaptation, mutations, transformation into metabolically inactive forms, etc.) to cells had been experienced during the Earth's evolution? Is it appropriate to suggest that an exclusively single source of homogenous cell culture started biological evolution on Earth, or were there several physiologically different types of cell cultures that interacted and developed into present day life? So, who was the first ancestor from the whole diverse extremophilic microbial world that we observe today? If the formation of any planet required a certain high-temperature regime then it is obvious, the hyperthermophilic microorganisms have priority as the role of ancestors. Psychrophily would have to have developed after a significant temperature drop on the planet surface. Free oxygen at the formation and cooling of the planet is excluded, and this means that anaerobic types of metabolisms would have been primary. Acidophily probably was the initial phase in the biological history on Earth, and only after certain mineral precipitation and enough buffer concentration of CO_2 in the air the occurrence of alkaliphily became possible. Halophily perhaps developed as the latest stage when the arid climate dominated on the land masses of Earth. Due to the tiny sizes of bacterial and archaean cells, the pressure does not have a significant influence on them as it has for the highly organized forms of life with macroscopic sizes, but still the barophily was formed among microorganisms of deep-sea and deep-underground ecosystems. Another fundamental question in biology arises: How is life distributed after the endogenous origin of life on Earth or the delivery of microbial life from exogenous sources in the Cosmos? Was it from the surface to center, or opposite, from the center (initial requirement of high pressure and absence of light) to a surface?

The sequence and direction of mutations that allowed adaptations to occur in ancestor cells in response to the new changes of environments had a complicated character. It included not only multi-factor influence, but probably, back and forward vectors as well. During the process of evolution, biological cells could have experienced several extreme physico-chemical factors at the same time. As a result, newly formed organisms appeared that were resistant, tolerant, or even developed obligate requirement (phily) to the multiple aggressive environmental factors. This is supported by the fact that now we observe the existence of acidophilic thermophiles, halo-alkaliphilic thermophiles, barophilic hyperthermophiles, halo- or alkaliphilic psychrophiles, etc. The genetics of extremophilic microorganisms could be read as the map of environmental effects in the past and probably have enough flexibility for providing a necessary "buffer pool" towards the future stressful changes. In any case, life would easily become extinct at any global cataclysms on climatic and geophysical levels. Except for the environmental influence of the ecosystems affecting living cells, another very important factor was developed and designated as *biological*. Life itself even on the level of monotypic or pure culture always has competitive relations between cells: those that eat more and breath better, are the strongest and predominant, and the weaker forms die out. On the interspecies level this is expressed even more effectively: the specific behavior and physiological specifics such as an excretion of antibiotics, poisons, or an inhibition by trivial end metabolic products, all of these quite limit the neighborhood of surrounding species, that led to the creation of the adjusted functionally and physiologically microbial consortia, and then communities. Symbiotic relations between different kinds of microbial organisms are reflected on the cytological and genetic levels of eukaryotic organisms (mitochondria and chloroplasts) for energetic efficiency, and also in such phenomenon as the binary microbial cultures (cases when microbiologists cannot separate cells of two symbiotically (undetected trace compounds or an exchange by vitamins) related microbial cultures. It is quite possible that such relationships initially have also been developed in extreme environments.

So, the interaction of growing and accumulating organisms with the environment and other biological species consequently led to the creation of balanced ecosystems. All geopaleontological deposits represent an encoded chronology of biological activity. The formation of minerals and fossils is the direct or indirect result of biological activity. Fossilized carbon, oil, elemental sulfur, methane, gold, iron pyrites, etc., all of them were formed in certain periods of evolution under specific circumstances, where local or wide-spread regions had experienced the demographic explosion of certain extremophilic populations, and the formation of rocks and minerals in such regions occurred due to the bioleaching processes or just to pure metabolic activity. After the global climatic changes and the geo-formation processes some populations with unique physiology were completely replaced by others forever, this can be observed on mineral and rock deposits. For this reason, all modern extremophiles could be appropriately called relics, because they do have genome that memorized environmental history, and this feature makes the study of extremophiles important to many fundamental sciences. Indeed, the modern face of the Earth is a product of the interaction of biological agents (mostly microorganisms) and minerals. Soil formation was the key moment for the distribution of plants on Earth, and it still remains an unsolved problem in biology. In this process, again the extremophilic microorganisms probably played a pioneering role. It is now well established that eukaryotic organisms cannot exist without prokaryotes; it is equally true for both plants (rhizosphere of root system, protective surface flora) and animals

(flora of intestine and skin surfaces). The participation of extremophilic bacteria in such ecosystems is not excluded (bacterium developing at extreme acidity in the stomach resulting in chronic disease, microbial process of methanogenesis in the intestine, etc.).

So, what is the situation with the cultivation of extremophilic microorganisms today? The molecular biologists have pronounced that microbiologists have successfully cultivated *in vitro* only one percent (or less) of all microbial diversity that exists in nature. Based on the data of genomic libraries constructed by 16 S rRNA gene sequence analysis, approximately 99% of whole gene pool of prokaryotes still remains unknown. At the present time around 7300 species of bacteria and archaea are validly published. From the time when Dr. Petri introduced the method of cultivation on agar solid media using dishes, which are now named for him, one hundred years have passed, but hyperthermophilic anaerobes were discovered only in the past twenty five years or so. Of course, the percent of cultivated extremophilic microorganisms compared to other "normal" bacteria and archaea is significantly lower. The development of biotechnology has tremendously stimulated the investigation of extremophiles. Today many laboratories in different countries are extensively engaged in the study of these fascinating and critically important microorganisms.

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