Microbial Diversity and Bioprospecting
Edited by Alan T. Bull

Chapter 14

Extremophiles: pH, Temperature, and Salinity

CONSTANTINOS E. VORGIAS AND GARABED ANTRANIKIAN

From the anthropocentric point of view, microorganisms that are able to survive and grow optimally at temperatures below 10°C and above 40°C, at a pH below 5.0 or above 8.0, at a pressure above 1 atm, and at a salt concentration of more than 30 g/liter are defined as extremophiles. There are many environments around the world that are extreme. These are geothermal areas with high temperature, polar regions with temperatures around the freezing point of water, deep oceans with very high pressure, and acid or alkaline springs with low or high pH, respectively. As conditions become increasingly demanding, extreme environments become exclusively populated by microorganisms belonging to prokaryotes. It is very likely that eukaryotic organisms were partially unable to adapt and survive under extreme conditions because of their cellular complexity. The realization that extreme environments harbor different kinds of prokaryote lineages has resulted in a complete reassessment of our concept of microbial evolution and has given considerable impetus to extremophile research (Leuschner and Antranikian, 1995; Ladenstein and Antranikian, 1998; Niehaus et al., 1999; Horikoshi and Grant, 1998, Rohschild and Mancmelli, 2001).

During the past 20 years, rapidly growing research activities focused on the elucidation of the basic rules that govern these extreme microorganisms have been conducted all over the world. This new field was strongly supported by industry and academia because it became obvious that the extremophilic organisms provide a unique resource for a variety of biomolecules such as enzymes and compounds with high potential for applications in the biotechnological industry. Furthermore, the finding of novel biocatalysts will allow the development of more efficient and environmentally friendly industrial processes. In this chapter we focus on a general description of the ecology, the general properties, and some examples of the biotechnological application of microorganisms that are able to grow optimally under very low or very high temperature, extreme pH, and high salinity. Such extremophiles can be found in terrestrial and marine environments all over the world and particularly in exotic ecological niches such as polar regions, solfataric fields, soda lakes, and abyssal hypothermal vents.

MICROORGANISMS LIVING AT EXTREME PH

Acidophiles

Solfataric fields are the most common biotopes for microorganisms that prefer to live at high temperature and acidic conditions. Solfataric soils consist of two layers with distinguished features. The upper layer is aerobic and has an ochre color that is due to the presence of ferric iron, and the lower layer is anaerobic and appears rather blackish-blue due to the presence of ferrous iron. The chemical composition of the two layers determines the variety of microorganisms that dominate in this environment.

Thermophilic acidophiles belonging to the genera *Sulfolobus, Acidianus, Thermoplasma,* and *Picrophilus,* with growth optima between 60 and 90°C and a pH of 0.7 to 5.0, are usually found in the aerobic upper layer of solfataric fields. These microorganisms maintain their intracellular pH between 4 and 6.5. On the other hand, slightly acidophilic or neutrophilic anaerobes such as *Thermoproteus tenax* or *Methanothermus fervidus* have been isolated from the lower layer of solfataric fields. *Thermoplasma* spp. (growth optima, pH 2 and 60°C) have been found in hot springs, solfataras, and coal refuse piles (Horikoshi and Grant, 1998). While their phylogenetic relatives *Picrophilus* spp. have been found in

---

Constantinos E. Vorgias • National and Kapodistrian University of Athens, Faculty of Biology, Department of Biochemistry-Molecular Biology, Panepistimiopolis-Zographou, 15701 Athens, Greece. **Garabed Antranikian** • Technical University Hamburg-Harburg, Technical Microbiology, Kasernenstrasse 12, 21073 Hamburg, Germany.

solfataras and are so far the most extreme acidophiles because they grow at a pH close to 0, *P. oshimae* and *P. torridus* are both aerobic, heterotrophic *Archaea* that grow optimally at 60°C and pH 0.7 and utilize various biopolymers such as starch and proteins as carbon sources.

Members of the genus *Sulfolobus* are strict aerobes growing either autotrophically, heterotrophically, or facultative heterotrophically. During autotrophic growth, S^0, S^{2-}, and H_2 are oxidized to sulfuric acid or water as end products. *Sulfolobus metallicus* and *S. brierley* are able to grow by oxidation of sulfidic ores. A dense biofilm of these microorganisms is responsible for the microbial ore leaching process, in which heavy metal ions such as Fe^{2+}, Zn^{2+}, and Cu^{2+} are solubilized. Several hyperthermophilic acidophiles have been assigned to the genera *Metallosphaera* (growth range, 50 to 80°C, pH 1 to 4.5), *Acidianus* (growth range, 60 to 95°C, pH 1.5 to 5), and *Stygioglobus* (growth range, 57 to 89°C, pH 1 to 5.5) (Stetter, 1996) and to *Thiobacillus caldus* (T_{max}, 55°C), isolated by Hallberg and Lindström (1994).

Alkaliphiles

Alkaliphiles, which grow at high pH values, are widely distributed throughout the world and require alkaline environments and sodium ions for growth. They have been found in carbonate-rich springs and alkaline soils, where the pH is usually 10.0 or higher, whereas their intracellular pH is maintained around 8.0. In these places, several species of cyanobacteria and *Bacillus* are normally abundant and provide organic matter for diverse groups of heterotrophs. Sodium ion-dependent uptake of nutrients has been reported in alkaliphiles. Many alkaliphiles require various nutrients for growth. A few alkaliphilic *Bacillus* strains can grow in simple minimal media containing glycerol, glutamic acid, and citric acid (Horikoshi and Grant, 1998). In general, the cultivation temperature is in the range of 20 to 55°C.

Haloalkaliphiles are microorganisms that have been isolated from alkaline hypersaline lakes and can grow in alkaline media containing 20% NaCl. A typical habitat where alkaliphilic microorganisms can be isolated is the soda lakes in the Rift Valley of Kenya. Similar lakes have been found in a few other places on Earth that are highly alkaline with pH values between 11.0 and 12.0.

Two thermoalkaliphilic bacteria, *Anaerobranca gottschalkii* and *Anaerobranca horikoshii*, have been isolated from Lake Bogoria in Kenya and from Yellowstone National Park, respectively. The new isolates represent a new line within the *Clostridium*/*Bacillus* subphylum (Prowe and Antranikian, 2001; Wiegel, 1998). The two archaeal thermoalkaliphiles identified to date are *Thermococcus alcaliphilus* and *Thermococcus acidoaminivorans*, both growing at 85°C and with a pH of 9.0. Table 1 summarizes the most studied acidophilic and alkaliphilic microorganisms.

Table 1. Microorganisms living at extreme pHs

Microorganisms	Optimal growth	
	°C	pH
Acidophilic microorganisms		
Sarcina ventriculi	37	4.0
Thiobacillus ferrooxidans	37	2.5
Alicyclobacillus acidocaldarius	55	2.0–6.0
Picrophilus oshimae	60	0.7
Picrophilus torridus	60	0.7
Thermoplasma acidophilum	60	2.0
Sulfolobus acidocaldarius	75	2.5
Acidianus infernus	75	2.0
Alkaliphilic microorganisms		
Many cyanobacteria	(25–37)	6.0–8.0
Spirulina spp.		8.0–10.0
Chromatium sp.		8.5
Bacillus spp.		11.5
Anaerobranca gottschalkii	55	9.5
Thermococcus alcaliphilus	85	9.0
Thermococcus acidoaminivorans	85	9.0

MICROORGANISMS GROWING AROUND THE FREEZING POINT OF WATER

Many parts of the world rarely reach temperatures above 5°C, and numerous bacteria, yeast, unicellular algae, and fungi have successfully colonized cold environments (see chapter 13). These psychrophilic microorganisms are able to grow at temperatures around 0°C and have developed adaptation mechanisms at various levels (enzymes, membranes, etc.). Most of the cold-adapted microorganisms have been isolated and characterized from the Arctic and Antarctic seawaters. It is noteworthy that, despite the harsh conditions, the density of bacterial cells in the Antarctic oceans is as high as the regular density in temperate waters (Deming, 2002; Russel and Hamamoto, 1998).

The microorganisms that are able to grow at or close to the freezing point of water can be divided into two main groups: psychrophiles and psychrotolerants.

Psychrophilic microorganisms are defined by an optimum temperature for growth at about 15°C, a maximum growth temperature at about 20°C, and a minimum temperature for growth at 0°C or lower. Psychrophiles can be found in permanently cold environments such as the deep sea, glaciers, mountain regions, or soils, and in fresh or saline waters associated with cold-blooded animals such as fish or crustaceans. Psychrotolerant microorganisms generally do not grow at 0°C and have optimum and maximum growth temperatures above 20°C (Morita, 1975). In general, psychrophiles have significantly narrower growth temperature ranges and lower optimum or maximum growth temperatures compared with psychrotolerant microorganisms.

Because psychrotolerant microorganisms are able to grow above 20°C, they could be pathogenic for humans and animals. A notable example is *Listeria monocytogenes,* which has a broad growth temperature between 0 and 40°C. Several other psychrophilic microorganisms are pathogenic, such as *Yersinia enterocolitica* and *Erwinia* spp. Several food-spoilage psychrotolerant bacteria have been identified, including *Brochothrix thermosphacta, Pseudomonas fragi, Bacillus cereus,* and *Clostridium botulinum.* Table 2 lists a few selected psychrophilic microorganisms.

The current studies on psychrophilic microorganisms are not so extensive compared with the other extremophiles. The biotechnological potential of psychrophiles has not fully emerged, and it is necessary to stress efforts to this area in order to fill this gap.

A systematic investigation has been undertaken to describe the microbial diversity and to quantify the microorganism in cold environments. Most of the studies, based on molecular biology techniques, have been concentrated on benthic communities while the sea-ice ecosystems have been relatively neglected (but see chapter 13). Bowman and Bowman (2001) have investigated bacterial diversity in sea ice using direct cloning of 16S rDNA sequences amplified from sea-ice DNA. Most of the bacteria identified were classified in four phylogenetic groups: *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria,* and the *Cytophaga-Flavobacterium-Bacteroides* division, as were supported by cultivation data. Many clones detected in these groups demonstrated high similarity to cultured strains isolated from Arctic or Antarctic sea ice or from other polar habitats. Surprisingly, no *Archaea* were detected using the universal archaeal-specific primers in any of the investigated sea-ice samples.

Table 2. Microorganisms growing at low temperature

Microorganisms	Optimal growth(°C)
Vibrio sp.	<20
Micrococcus criophilus	<20
Arthrobacter glacialis	<20
Vibrio psychroerythreus	<20
Carnobacterium sp.	<20
Antarctic strain TABS	<20
Alteromonas haloplanctis A23	<20
Moritella marina	<20
Psychrobacter sp. TA137	<20
Bacillus psychrosaccharolyticus	<20
Moraxella sp. TA144	<20
Psychrobacter immobilis B10	<20
Aquaspirillum arcticum	<20

At the biochemical level, a systematic investigation has been carried out in order to understand the rules governing the molecular mechanisms for adaptation to low temperatures. These fundamental aspects are closely associated with a strong biotechnological interest aimed at the exploitation of these microorganisms and their cell components such as membranes, polysaccharides, and enzymes (Russell, 2000).

MICROORGANISMS GROWING AROUND THE BOILING POINT OF WATER

Hyperthermophilic microorganisms are adapted to grow optimally at high temperatures (60 to 108°C) and have been isolated from their most common biotopes, i.e., volcanic and hydrothermal vent systems, solfataric fields, neutral hot springs, and submarine hot vents. The most interesting are submarine hydrothermal systems that are situated in shallow and abyssal depths. They consist of hot fumaroles, springs, sediments, and deep-sea vents with temperatures up to 400°C ("black smokers") (Stetter, 1996). Shallow marine hydrothermal systems have been detected at the beaches of Vulcano, Naples, and Ischia (Italy); Sao Miguel (Azores); and Djibouti (Africa). The best-studied deep-sea hydrothermal systems are (i) the Guaymas Basin (depth, 1,500 m) and the East Pacific Rise (depth, 2,500 m), both off the coast of Mexico; (ii) the Mid-Atlantic Ridge (depth, 3,700 m); and (c) the Okinawa Trough (depth, 1,400 m) (Grote et al., 1999; Jeanthon et al., 1999, 1998; Gonzáles et al., 1998; Canganella et al., 1998).

The genera *Pyrococcus, Pyrodictium, Igneococcus, Thermococcus, Methanococcus, Archaeoglobus,* and *Thermotoga* are the major members of the shallow as well as deep-sea hydrothermal systems (Jeanthon et al., 1998). So far, members of the genus *Methanopyrus* have been found only at greater depths, whereas *Aquifex* was isolated exclusively from shallow hydrothermal vents (Stetter, 1998). Recently, interesting biotopes of extreme and hyperthermophiles were discovered in deep, geothermally heated oil reservoirs around 3,500 m below the bed of the North Sea and the permafrost soil of north Alaska (Lien et al., 1998; Stetter et al., 1983).

Moderate thermophiles are microorganisms that are capable of growing optimally at temperatures between 50 and 60°C. Most of these microorganisms belong to many different taxonomic groups of eukaryotic and prokaryotic microorganisms such as protozoa, fungi, algae, streptomycetes, and cyanobacteria, which comprise mainly mesophilic species. It can be assumed that moderate thermophiles, which are closely related phylogenetically to mesophilic organisms, may be secondarily adapted to life in hot environments. Extreme thermophiles, which grow optimally between 60 and 80°C, are widely distributed among the genera *Bacillus, Clostridium, Thermoanaerobacter, Thermus, Fervidobacterium,* and *Thermotoga.*

Hyperthermophiles are represented among all the deepest and shortest lineages, including the genera *Aquifex* and *Thermotoga* within the *Bacteria* and *Pyrodictium, Pyrobaculum, Thermoproteus, Desulfurococcus, Sulfolobus, Methanopyrus, Pyrococcus, Thermococcus, Methanococcus,* and *Archaeoglobus* within the *Archaea.* Interestingly, the majority of the hyperthermophiles isolated to date belong to the archaeal domain of life, and no eukaryotic organism has been found that can grow at the boiling point of water.

The relative abundance of *Archaea* and *Bacteria* in high-temperature environments was, until recently, mainly studied by cultivation-based techniques. Because of the frequent isolation of *Archaea* from these habitats, it was assumed that Archaea dominate the high-temperature biotopes (Baross and Deming, 1995; Stetter et al., 1990). Recently, the application of molecular biological methods revealed a quite different picture. Slot-blot hybridizations of rRNA utilizing oligonucleotide probes targeting the 16S rRNA of Archaea and Bacteria revealed that bacteria seem to be the major population of the microbial community along a thermal gradient at a shallow submarine hydrothermal vent near Milos Island, Greece (Sievert et al., 2000). Bacteria made up at least 78% (mean, 95%) of the prokaryotic rRNA. Along the steepest temperature gradient, the proportion of archaeal rRNA increased. Nevertheless, even in the hottest sediment layer, archaeal rRNA made up only around 12% of the prokaryotic rRNA. These results suggest that archaea may generally be of lower abundance in hot environments than could be assumed from cultivation-based experiments. However, the factors that allow bacteria to dominate in high-temperature habitats, that were once believed to be the territory of archaea, remain unknown. Most of these microorganisms that can be found in low-salinity and submarine environments are strict anaerobes. Terrestrial solfataric fields found in Italy or Iceland harbor members of the genera *Pyrobaculum, Thermoproteus, Thermofilum, Desulfurococcus,* and *Methanothermus. Pyrobaculum islandicum* and *Thermoproteus tenax* are able to grow chemolithoautotrophically, gaining energy by anaerobic reduction of S^0 by H_2. In contrast to these strictly anaerobic microorganisms, *Pyrobaculum aerophilum* and *Aeropyrum pernix* are able to use oxygen as a final electron acceptor. *Methanothermus fervidus,* on the other hand, is highly sensitive toward oxygen and can survive only in low-redox environments at temperatures between 65 and 97°C. Some microorganisms from marine environments such as members of the genera *Archaeoglobus, Methanococcus,* and *Methanopyrus* are able to grow chemolithoautotrophically, gaining energy by the reduction of SO_4^{2-} by H_2 (*Archaeoglobus lithothrophicus* and *A. fulgidus*) or by the reduction of CO_2 by H_2 (*Methanococcus jannaschii, Methanopyrus kandleri*). Other members of the hyperthermophilic genera *Staphylothermus, Pyrococcus, Thermococcus,* and *Pyrodictium* are adapted to marine environments (NaCl concentration of about 30 g/liter). Most of them gain energy by fermentation of polysaccharides, peptides, amino acids, and sugars (Stetter, 1996). Consequently, such thermophilic microorganisms have been found to be producers of interesting polymer-degrading enzymes of industrial relevance (Niehaus et al., 1999; Vorgias et al., 2000).

Among the bacterial domain of life, members of the genera *Aquifex* and *Thermotoga* represent the deepest phylogenetic branches. Within the latter genus, *Thermotoga maritima* and *T. neapolitana* are the most thermophilic species with a maximal growth temperature of about 90°C. The representatives of the order of *Thermotogales* also provide a resource of unique thermoactive enzymes. Some representative microorganisms living at high temperature are presented in Table 3.

Table 3. Microorganisms growing at elevated temperatures

Microorganisms	Optimal growth (°C)
Moderate thermophiles (50–60°C)	
Bacillus acidocaldarius	50
Bacillus stearothermophilus	55
Extreme thermophiles (60–80°C)	
Thermus aquaticus	70
Thermoanaerobacter ethanolicus	65
Clostridium thermusulfurogenes	60
Fervidobacterium pennivorans	75
Hyperthermophiles (80–110°C)	
Thermotoga maritima	90
Aquifex pyrophilus	85
Archeoglobus fulgidus	83
Methanopyrus kandleri	88
Sulfolobus sulfataricus	88
Thermococcus aggregans	88
Pyrobaculum islandicum	100
Pyrococcus furiosus	100
Pyrodictium occultum	105
Pyrolobus fumarii	106

MICROORGANISMS GROWING AT EXTREME SALINITY

Halophiles are *Bacteria* and *Archaea* that grow optimally at NaCl concentrations above the average concentration of seawater (>0.6 M NaCl). Generally, halophilic microorganisms are classified as moderate halophiles for those that can grow at salt concentrations between 0.4 and 3.5 M NaCl and as extreme halophiles for those that require NaCl concentrations above 2 M for growth (Grant et al., 1998). Halophiles have been mainly isolated from saline lakes, such as the Great Salt Lake in Utah (salinity, >2.6 M) and from evaporitic lagoons and coastal salterns with NaCl concentrations between 1 and 2.6 M (Grant et al., 1998). Saline soils are less well explored. Bulk salinity measurements of 1.7 to 3.4 M NaCl have been reported for saltern soils (Ventosa and Bieto, 1995). Saline soils constitute less-stable biotopes than hypersaline waters because they are subjected to periodic significant dilution during rainy periods. There is no doubt that almost all hypersaline habitats harbor significant populations of specifically adapted microorganisms. However, it remains unclear what substrates might be available for growth in these biotopes. Hypersaline lakes often contain up to 1 g of dissolved organic carbon per liter. In many of these lakes, primary producers such as cyanobacteria, anoxygenic phototrophic bacteria, and algae may be the main sources of organic compounds (Grant et al., 1998; Jones et al., 1998; Kamekura, 1998).

In a study of aerobic heterotrophs in a marine saltern it has been shown that bacterial halophiles were predominant up to 2 M NaCl. Above this concentration, archaeal halophiles become predominant, almost to the exclusion of *Bacteria.* Halophilic primary producers mainly belong to the cyanobacteria and anoxygenic phototrophic sulfurbacteria. The former often thrive in eutrophic salterns, forming large floating mats. The latter group, on the other hand, grows either in anaerobic sediments or in the water column where they are responsible for the characteristic red color of high-salinity habitats. The range of heterotrophic *Bacteria* comprises proteobacteria, actinomycetes, and gram-positive rods and cocci. Fermentative anaerobes as well as sulfur oxidizers, sulfate reducers, and nitrate reducers are also present and give rise to the assumption that all kind of metabolic features can be found in high-salinity environments. Halophilic bacteria do not belong to one homogeneous group but rather fall into many bacterial taxa in which the capability to grow at high salt concentrations is a secondary adaptation.

The term "halobacteria" refers to the red-pigmented, extremely halophilic *Archaea,* members of the family *Halobacteriaceae* and the only family in the order *Halobacteriales* (Ventosa and Bieto, 1995). Most halobacteria require 1.5 M NaCl in order to grow and to retain the structural integrity of the cell. Halobacteria can be distinguished from halophilic bacteria by their archaeal characteristics, in particular the presence of ether-linked lipids (Ross et al., 1981). Most halobacteria are colored red or orange due to the presence of carotenoids, but some species are colorless, and those with gas vesicles form opaque, white, or pink colonies. A purple hue can be seen in halobacteria that form the bacteriorhodopsin-containing purple membrane (Grant et al., 1998). Halobacteria are the most halophilic organisms known so far and form the dominant microbial population when hypersaline waters approach saturation (Ventosa et al., 1998). Interestingly, the reddening caused by halobacterial blooms has an impact on the evaporation rates in salterns. It is known that the carotenoid pigments of halobacteria trap solar radiation, thus increasing the ambient temperature and evaporation rates (Madigan and Oren, 1999). Some representative microorganisms living at high salt concentration are listed in Table 4.

The singular physiology of halophilic microorganisms that have to cope with 4 M ion concentration inside and outside the cell has evolved potentially interesting enzymes that can function under conditions of low-water activity that could be imposed by compounds other than salts, for example, solvents. Interestingly, halophilic and marine halo-

Table 4. Microorganisms living at high salt concentrations

Halophilic microorganisms	NaCl concentration (M) required for growth		
	Maximum	Minimum	Optimum
Dunaliella spp.	0.3		5.0
Clostridium halophilum	0.15	0.6	6.0
Haloanaerobium praevalens	0.8	2.2	4.3
Halobacterium sp.		2.0	5.5
Halobacterium denitrificans	1.5	2.5	4.5
Haloferax vulcanii	1.0	1.5	3.0
Methanohalobium evestigatum			4.3

tolerant bacteria produce and/or accumulate organic osmolytes (compatible solutes, e.g., ectoin, hydroxyectoin, trehalose) for osmotic equilibrium. These metabolically compatible hygroscopic compounds not only protect living cells in a low-water environment but also exhibit an enzyme-stabilizing effect in vitro against a variety of stress factors such as heating, freezing, urea, and other denaturants (Margesin and Schinner, 2001).

BIOCATALYSIS UNDER EXTREME CONDITIONS

Extremophiles in general have been considered as a group of microorganisms with biotechnological potential. It is clear that hyperthermophiles represent a huge, almost unexplored potential for novel applications in modern biotechnology. Applications of hyperthermophiles in biotechnology are in an early phase of growth. At present there are several known examples but these represent only a small portion of the real potential of extremophilic biomolecules (Herbert, 1992). A breakthrough in applications of hyperthermophilic enzymes occurred in the late 1980s with the application of heat-stable DNA polymerase for DNA amplification by PCR. This was a milestone in the field of basic and applied biological research (Frey and Suppmann, 1995). Several thermophilic hydrolases, such as proteases, lipases, amylases, and xylanases, have the potential to be incorporated in existing industrial processes toward a more economical and environmentally friendly procedure (Sunna et al., 1997).

Several enzymes from hyperthermophiles have been purified and characterized (Niehaus et al., 1999; Vorgias et al., 2000). As a general rule, they show an extraordinary heat stability even in vitro. *Pyrococcus woesei* harbors an amylase that is active even at 130°C for more than 30 min (Koch et al., 1991). Within the bacterial domain, *Thermotoga maritima* MSB8 possesses an extremely heat-resistant, membrane-associated xylanase that is optimally active at 105°C (Winterhalter and Liebl, 1995). Even complex enzymes such as DNA-dependent RNA polymerases or glutamate dehydrogenases show a remarkable heat stability.

Because extremophilic microorganisms are able to survive under unusual conditions, it is clear that they have to develop strategies to withstand these conditions. For the case of the extreme thermophilic organisms, the cell components have to be either intrinsically heat resistant or stabilized by other components within the cells. The molecular basis of thermostability is a very exciting and difficult issue and is still under intensive investigation (Ladenstein and Antranikian, 1998; Stetter, 1998; Vetriani et al., 1998). Up to now a solid mechanistic basis for thermostabilization has been achieved, and one is able to understand but not yet predict thermostabilization mechanisms. The basic principles of heat stabilization of several thermostable enzymes have been established, and it has become clear that each protein has found its own strategy for stabilization through the evolution (Jaenicke and Bohm, 1998).

The best known example of a biotechnological product derived from the studies on halophiles is the small molecules known as compatible solutes. Halophilic and/or halotolerant bacteria produce, among others, the ectoine-type osmolytes (2-methyl-1,4,5,6-tetrahydropyrimidine derivatives) that represent the most abundant class of stabilizing solutes (De Costa et al., 1998). The extrinsic stabilization effect of ectoines and other compatible solutes is most likely based on solvent-modulating properties of these compounds. Osmolytes have considerable potential as effective stabilizers for the hydration shell of proteins and hence could be highly efficient against stress conditions for biomolecules and therefore stabilizers that might be suitable for sensitive vaccines or industrial enzymes functioning under extreme conditions (Galinski, 1993). Recently, it has been shown that several hyperthermophilic microorganisms are also able to produce a variety of compatible solutes that were found to be effective in enzyme stabilization (De Costa et al., 1998). Because halophiles can work at high salt concentrations (up to 4 M), their enzymes might be capable of working under low-water conditions and open the possibility to function in nonaquatic solvents. Although there is considerable interest in halophiles, to date they have little impact in the commercial field.

The main industrial application of alkaliphilic enzymes is in the detergent industry, which accounts for nearly 30% of the total worldwide enzyme production. Alkaline enzymes have been used in the hide-dehairing process that is usually carried out at a pH between 8 and 10. Several other alkaliphilic hydrolases such as amylases, xylanases, pullulanases, and cyclomaltodextrin glucanotransferases (CGTases) have been isolated and are under intensive investigation for their potential industrial application (Horikoshi and Grant, 1998).

The possible applications of cold-active enzymes are as detergents for cold washing (proteases, lipases, cellulases) and food additives such as polyunsaturated flavor-modifying agents. In the dairy industry, β-galactosidase is applied to reduce the amount of lactose in milk, which is responsible for lactose intolerance in approximately two-thirds of the world's population. The clarification of fruit juices is achieved by the addition of cold-active pectinases. Further applications of cold-active enzymes are found in food processing such as cheese manufacturing; tenderizing meat by proteases; and improving the baking process by the addition of amylases, proteases, and xylanases. Cold-active enzymes are also used in biosensors for environmental applications and for cleaning contact lenses (Russell and Hamamoto, 1998).

Some of the advantages of using psychrophiles and their enzymes in biotechnological applications are the rapid termination of the process by moderate heat treatment, higher yields of thermosensitive components, modulation of the (stereo-) specificity of enzyme-catalyzed reactions, cost saving by elimination of expensive heating and cooling process steps, and finally the capacity for online monitoring under environmental conditions (Gerday et al., 2000; Feller et al., 1996).

REFERENCES

Baross, J. A., and J. W. Deming. 1995. Growth at high temperatures: isolation and taxonomy, physiology, and ecology, p. 169–217. *In* D. M. Karl (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents.* CRC Press, Boca Raton, Fla.

Bowman, M. V., and J. P. Bowman. 2001. A molecular phylogenetic survey of sea-ice microbial communities. *FEMS Microbiol. Ecol.* 35:267–275.

Canganella, F., W. J. Jones, A. Gambacorta, and G. Antranikian. 1998. *Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site. *Int. J. Syst. Bacteriol.* 48:1181–1185.

De Costa, M. S., H. Santos, and E. A. Galinski. 1998. An overview of the role and diversity of compatible solutes in Bacteria and Archaea. *Adv. Biochem. Eng. Biotechnol.* 61:117–153.

Deming, J. W. 2002. Psychrophiles and polar regions. *Curr. Opin. Microbiol.* 5:301–309.

Demirjian, D. C., F. Moris-Varas, and C. S. Cassidy. 2001. Enzymes from extremophiles. *Curr. Opin. Chem. Biol.* 5:144–151.

Feller, G., E. Narinx, J. L. Arpigny, M. Aittaleb, E. Baise, S. Genicot, and C. Gerday. 1996. Enzyme from extremophilic mocroorganisms. *FEMS Microbiol. Rev.* 18:189–202.

Frey, B., and B. Suppmann. 1995. Demonstration of the Expand PCR System's greater fidelity and higher yields with a lacI-based fidelity assay. *Biochemica* 2:34–35.

Galinski, E. A. 1993. Compatible solutes of halophilic eubacteria: molecular principles, water-soluble interactions, stress protection. *Experientia* 49:487–496.

Gerday, C., M. Aittaleb, M. Bentahir, J. P. Chessa, P. Claverie, T. Collins, S. D'Amico, J. Dumont, G. Garsoux, D. Georlette, A. Hoyoux, T. Lonhienne, M. A. Meuwis, and G. Feller. 2000. Cold-adapted enzymes: from fundamentals to biotechnology. *Trends Biotechnol.* 18:103–107.

Gonzáles, J. M., Y. Masuchi, F. T. Robb, J. W. Ammerman, D. L. Maeder, M. Yanagibayashi, J. Tamaoka, and C. Kato. 1998. *Pyrococcus horikoshii* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. *Extremophiles* 2:123–130.

Grant, W. D., R. T. Gemmell, and T. J. McGenity. 1998. Halophiles, p. 93–133. *In* K. Horikoshi and W. D. Grant (ed.), *Halophiles.* Wiley-Liss, New York, N.Y.

Grote, R., L. Li, J. Tamoaoka, C. Kato, K. Horikoshi, and G. Antranikian. 1999. *Thermococcus siculi* sp. nov., a novel hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Okinawa Trough. *Extremophiles* 3:55–62.

Hallberg, K. B., and E. B. Lindström. 1994. Characterization of *Thiobacillus caldus* sp. nov., a moderately thermophilic acidophile. *Microbiology* 140:3451–3456.

Herbert, R. A. 1992. A perspective on the biotechnological potential of extremophiles. *Trends Biotechnol.* 110:395–402.

Horikoshi, K., and W. D. Grant (ed.). 1998. *Extremophiles—Microbial Life in Extreme Environments.* Wiley-Liss, New York, N.Y.

Jaenicke R, and G. Bohm. 1998. The stability of proteins in extreme environments. *Curr. Opin. Struct. Biol.* 8:738–748.

Jeanthon, C., S. L'Haridon, A. L. Reysenbach, M. Vernet, P. Messner, U. B. Sleytr, and D. Prieur. 1998. *Methanococcus infernus* sp. nov., a novel hyperthermophilic lithotrophic methanogen isolated from a deep-sea hydrothermal vent. *Int. J. Syst. Bacteriol.* 48:913–919.

Jeanthon, C., S. L.'Haridon, A. L. Reysenbach, E. Corre, M. Vernet, P. Messner, U. B. Sleytr, and D. Prieur. 1999. *Methanococcus vulcanius* sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise, and identification of *Methanococcus* sp. DSM 4213T as *Methanococcus fervens* sp. nov. *Int. J. Syst. Bacteriol.* 49:583–589.

Jones, B. E., W. D., Grant, A. W. Duckworth, and G. G. Owenson. 1998. Microbial diversity of soda lakes. *Extremophiles* 2:191–200.

Kamekura, M. 1998. Diversity of extremely halophilic bacteria. *Extremophiles* 2:289–295.

Koch, R., K. Spreinat, K. Lemke, and G. Antranikian. 1991. Purification and properties of a hyperthermoactive alpha-amylase from the archaeobaterium *Pyrococcus woesei. Arch. Microbiol.* 155:572–578.

Ladenstein, R., and G. Antranikian. 1998. Proteins from hyperthermophiles: stability and enzymatic catalysis close to the boiling point of water. *Adv. Biochem. Eng. Biotechnol.* 61: 37–85.

Leuschner, C., and G. Antranikian. 1995. Heat-stable enzymes from extremely thermophilic and hyperthermophilic microorganisms. *World Microbiol. Biotechnol.* 11:95–114.

Lien, T., M. Madsen, F. A. Rainey, and N. K. Birkeland. 1998. *Petrotoga mobilis* sp. nov., from a North Sea oil-production well. *Int. J. Syst. Bacteriol.* 48:1007–1013.

Madigan, M. T., and A. Oren. 1999. Thermophilic and halophilic extremophiles. *Curr. Opin. Microbiol.* 2:265–269.

Margesin, R., and F. Schinner. 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles* 5:73–83.

Morita, R. Y. 1975. Psychrophilic bacteria. *Bacteriol. Rev.* 39: 144–167.

Niehaus, F., C. Bertoldo, M. Kahler, and G. Antranikian. 1999. Extremophiles as a source of novel enzymes for industrial application. *Appl. Microbiol. Biotechnol.* 51:711–729.

Prowe, S. G., and G. Antranikian. 2001. *Anaerobrances gottschalkii* sp. nov., a new hermoalkaliphilic bacterium that grows anaerobically at high pH and temperature. *Int. J. Syst. Evol. Microbiol.* 51:457–465.

Ross, H. N. M., M. D. Collins, B. J. Tindall, and W. D. Grant. 1981. A rapid method for detection of archaeabacterial lipids in halophilic bacteria. *J. Gen. Microbiol.* 123:75–80.

Rothschild, L. J., and R. L. Mancinelli. 2001. Life in extreme environments. *Nature* 22:1092–1101.

Russell, N. J. 2000. Toward a molecular understanding of cold activity of enzymes from psychrophiles. *Extremophiles* 4:83–90.

Russell, N. J. and T. Hamamoto. 1998. Psychrophiles, p. 25–47. *In* K. Horikoshi and W. D. Grant (ed.), *Extremophiles—Microbial Life in Extreme Environments,* Wiley-Liss, New York, N.Y.

Sievert, S. M., W. Ziebis, J. Kuever, and K. Sahm. 2000. Relative abundance of Archaea and Bacteria along a thermal gradient of a shallow-water hydrothermal vent quantified by rRNA slot-blot hybridization. *Microbiol* 146:1287–1293.

Stetter, K. O. 1996. Hyperthermophilic procaryotes. *FEMS Microbiol. Rev.* 18:149–158.

Stetter, K. O. 1998. Hyperthermophiles: isolation, classification and properties, p. 1–24. *In* K. Horikoshi and W. D. Grant (ed.), *Extremophiles—Microbial Life in Extreme Environments.* Wiley-Liss, New York, N.Y.

Stetter, K. O., H. König, and E. Stackebrandt. 1983. *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulfur reducing archaebacteria growing optimally at 105°C. *Syst. Appl. Microbiol.* 4:535–551.

Stetter, K. O., G. Fiala, G. Huber, R. Huber, and A. Segerer. 1990. Hyperthermophilic microorganisms. *FEMS Microbiol. Rev.* 75: 117–124.

Sunna, A., M. Moracci, M. Rossi, and G. Antranikian. 1997. Glycosyl hydrolases from hyperthermophiles. *Extremophiles* 1:2–13.

Ventosa, A., and J. J. Bieto. 1995. Biotechnological applications and potentialities of halophilic microorganisms. *World J. Microbiol. Technol.* 11:85–94.

Ventosa, A., M. C. Marquez, M. J. Garabito, and D. R. Arahal. 1998. Moderately halophilic gram-positive bacterial diversity in hypersaline environments. *Extremophiles* 2:297–304.

Vetriani, C., D. L. Maeder, N. Tolliday, K. S. P. Yip, T. J. Stillman, K. L. Britton, D. W. Rice, H. H. Klump, and F. T. Robb. 1998. Protein thermostability above 100°C: a key role for ionic interactions. *Proc. Nat. Acad. Sci. USA* 95:12300–12305.

Vorgias, C. E., and G. Antranikian. 2000. Glycosyl hydrolases from extremophiles, p. 313–339. *In* Doyle (ed.), *Glycomicrobiology.* Kluwer Academic, New York, N.Y.

Wiegel, J. 1998. Anaerobic alkalithermophiles, a novel group of extremophiles. *Extremophiles* 2:257–267.

Winterhalter, C., and W. Liebl. 1995. 2 Extremely thermostable xylamases of the hyperthermophilic bacterium *Thermotoga maritima* MSB8. *Appl. Environ. Microbiol.* 61:1810–1815.