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# The Biocatalytic Potential of Extremophiles and Extremozymes

*Joseph Gomes* and Walter Steiner*

Institute of Biotechnology and Bioprocess Engineering, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria

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**Summary**

Extremophiles are bizarre microorganisms that can grow and thrive in extreme environments, which were formerly considered too hostile to support life. The extreme conditions may be high or low temperature, high or low pH, high salinity, high metal concentrations, very low nutrient content, very low water activity, high radiation, high pressure and low oxygen tension. Some extremophiles are subject to multiple stress conditions. Extremophiles are structurally adapted at the molecular level to withstand these harsh conditions. The biocatalysts, called extremozymes, produced by these microorganisms, are proteins that function under extreme conditions. Due to their extreme stability, extremozymes offer new opportunities for biocatalysis and biotransformation. Examples of extremozymes include cellulases, amylases, xylanases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases, which have great potential for application in various biotechnological processes. Currently, only 1–2 % of the microorganisms on the Earth have been commercially exploited and amongst these there are only a few examples of extremophiles. However, the renewed interest that is currently emerging as a result of new developments in the cultivation and production of extremophiles and success in the cloning and expression of their genes in mesophilic hosts will increase the biocatalytic applications of extremozymes.

*Key words:* extremophiles, extremozymes, biocatalytic potential, thermophiles, psychrophiles, alkaliphiles, acidophiles, halophiles, piezophiles, metalophiles

## Introduction

Microorganisms requiring extreme environments for growth are called extremophiles and the enzymes they produce are called extremozymes. The term extremophile was first used by MacElroy in 1974, three decades ago (*1*). »Extreme environment« is a relative term, since environments that are extreme for one organism may be essential for the survival of another organism. Extremophiles thrive under conditions that would kill most other creatures and many cannot survive in the normal anthropogenic global environments. Extreme environments include those with either high (55 to 121 °C) or low (–2 to 20 °C) temperatures, high salinity (2–5 M NaCl) and either high alkalinity (pH>8) or high acidity (pH<4), reviewed by Madigan and Marrs (*2*) and Rothschild and Manicinelli (*3*). Various extremophiles can tolerate other extreme conditions including high pressure, high levels of radiation or toxic compounds, or conditions that we consider unusual, such as living in rocks deep below the surface of the earth or living in extremely dry areas with very low water and nutrient supply (*2,3*). In addition,

* Corresponding author; Phone: ++43 316 87 38 404; Fax: ++43 316 87 38 434; E-mail: gomes@biote.tu-graz.ac.at

extremophiles may be found in environments with a combination of extreme conditions such as high temperature and high acidity, high temperature and high alkalinity or high pressure and low temperature. The limits of temperature, pressure, pH, salinity and water activity at which life can thrive have not yet been precisely defined. Most of the extremophiles that have been identified to date belong to the domain of the archaea. However, many extremophiles from the eubacterial and eukaryotic kingdoms have also been identified recently and characterized (*2,3*).

The use of enzymes or microorganisms as biocatalysts for the formation of various products has been well documented in many publications and reviews (*4–12*). As industrial process conditions are harsh, there are demands for biocatalysts that can withstand the process conditions. The majority of the enzymes used to date originate from mesophilic organisms and, despite their many advantages, the application of these enzymes is restricted due to their limited stability at the extremes of temperature, pH and ionic strength. On the other hand, extremophiles are a potent source of extremozymes, which show outmost stability under extreme conditions. Consequently, much attention has been given to the microorganisms that are able to thrive in extreme environments. Thus, biocatalysis using extremophiles as well as extremozymes is rapidly being transformed from an academic science to an industrially viable technology. Each group of the extremophiles has unique features, which can be harnessed to provide enzymes with a wide range of application possibilities (*4–29*). Moreover, the dramatic advances in molecular and computational biology, new sources of enzymes, combinatorial methodologies and biochemical engineering of single and multicomponent enzyme systems have stimulated a renaissance in enzyme technology. Revolutionary changes in molecular biology are leading to the development of thousands of new uses of enzyme technology, fueling major growth in this multi-billion-dollar field. A huge amount of money is being invested worldwide in the development of industrial as well as biomedical applications of extremozymes and extremophiles.

In this review, selected examples of the discovery, isolation and application of extremophiles and their enzymes are discussed and presented. The fields of extremophiles and extremozymes have received a great deal of attention. The readers are also referred to some excellent reviews (*2–29*).

## Thermophiles

Thermophilic microorganisms have attracted most attention and are amongst the most studied of the extremophiles. The biocatalytic potential of thermophiles and their enzymes has been reviewed by Adams *et al.* (*4*), Hough and Danson (*5*), Niehaus *et al.* (*6*), Demirjian *et al.* (*7*), Van den Burg (*8*), Irwin and Baird (*9*), Eichler (*10*), Vieille and Zeikus (*11*), Haki and Rakshit (*12*), Fujiwara (*13*), Sellek and Chaudhuri (*14*), Lévêque *et al.* (*15*) and Bertoldo and Antranikian (*16*). Thermophiles can be generally classified into moderate thermophiles (growth optimum 50–60 °C), extreme thermophiles (growth optimum 60–80 °C) and hyperthermophiles (growth optimum 80–110 °C) (*3–15*). Among the extreme thermophiles, multicellular animals or plants cannot tolerate temperatures above about 50 °C and the eukaryotic microbes that have been discovered so far cannot survive at temperatures higher than 60 °C (*3*). Extreme thermophiles, growing optimally at 60–80 °C, are widely distributed among the genera *Bacillus*, *Clostridium*, *Thermoanaerobacter*, *Thermus*, *Fervidobacterium*, *Rhodothermus*, *Thermotoga* and *Aquifex* (*4–16*). On the other hand, most of hyperthermophiles belong to the archaea, which consists of four phyla: Crenarchaeota, Euryarchaeota, Korarchaeota and Nanoarchaeota. Some genera belonging to Crenarchaeota are: *Sulfolobus*, *Acidianus*, *Pyrodictium*, *Pyrolobus*, *Pyrobaculum*, *Desulfurococcus*, *Thermoproteus*, *Thermofilum* and *Staphylothermus* (*4–16*). Euryarchaeota include extreme halophiles (*e.g. Halobacterium*, *Halobaculum*, *Halococcus*, *Haloferax*, *Halorubrum*), methanogens (*e.g. Methanobacterium*, *Methanosphaera*, *Methanococcus*, *Methanobrevibacter* and *Methanothermus*), extreme acidophiles (*e.g. Picrophilus*, *Thermoplasma*) and extreme thermophiles (*e.g. Thermococcus*, *Pyrococcus*, *Methanopyrus*, *Archaeoglobus* and *Ferroglobus*) (*6–16*). The so-called phylum »Korarchaeota« has been postulated on the basis of PCR amplification of 16S rRNA genes from environmental DNA, but has not been confirmed by the pure cultivation of any organisms (*30*). The novel phylum Nanoarchaeota is represented by the hyperthermophilic anaerobic nano-sized coccus »*Nanoarchaeum equitans*«. It grows only in co-culture with a new chemolithoautotrophic *Ignicoccus* species (*31,32*). Among the hyperthermophiles, a strain dubbed »strain 121« (*33*) and *Pyrolobus fumarii* (*34*), capable of growing at 121 and 113 °C, respectively, hold the record for the most thermophilic microorganisms. These strains cannot grow below 90 °C. »Strain 121« grows chemoautotrophically using formate as an electron donor and $Fe^{3+}$, while *Pyrolobus fumarii* is a nitrate-reducing chemolithoautotroph. Thermophiles are more common than hyperthermophiles.

Short branches in the unrooted phylogenetic tree indicate a rather slow clock in the biological evolution and all hyperthermophiles that can grow at temperatures above 90 °C are represented as the deepest and shortest lineage, suggesting that hyperthermophiles are very primitive and their common ancestor is a hyperthermophile (*13,30*). The genome sizes of hyperthermophiles are comparable to those of mesophiles and are in fact often somewhat smaller. Generally, hyperthermophiles have a smaller size genome than well-studied mesophiles (*13,35*). The small size genomes of hyperthermophiles may define the lower limit of their genetic capacity. Chromosomes of hyperthermophiles are densely packed with genes, most of which are required for essential functions (*36*). This indicates that the earliest life forms may have had small genomes. The developments of functions and potential resources of extremophiles, with particular reference to gene isolation from natural environments without pure culturing, have been reviewed by Fujiwara (*13*).

Of all extremozymes, thermophilic enzymes have attracted most attention during the past four decades (*4–16*). Such enzymes are of great industrial and biotechnological interest due to the fact that the enzymes

are better suited for harsh industrial processes. There are many advantages of conducting industrial processes at high temperature, such as the increased solubility of many polymeric substrates, resulting in decreased viscosity, increased bioavailability, faster reaction rate and the decreased risk of microbial contamination. These enzymes have also been used as models for the understanding of thermostability and thermoactivity, which is useful for protein engineering (*37–39*). Structural features of thermophilic extremozymes have attracted much attention. Several three-dimensional structures have been resolved and compared with those of mesophilic counterparts, with the ultimate goal of elucidating the mechanisms underlying thermostability (*37,38*). A large number of sequence and structural factors are thought to contribute toward higher intrinsic thermal stability of proteins from thermophiles and hyperthermophiles. Thermophiles produce special proteins known as 'chaperonins', which are thermostable and resistant to denaturation and proteolysis. Proteins of thermophiles, denatured at high temperature, are refolded by the chaperonins, thus restoring their native form and function (*38,39*). The cell membrane of thermophiles consists of saturated fatty acids, which increase protein core hydrophobicity and keep the cell rigid enough to survive at high temperatures (*37–39*). Moreover, hyperthermophiles have membranes containing lipids linked with ether to their cell walls. This layer is much more heat resistant than a membrane formed of fatty acids. In addition, proteins of thermophiles have increased surface charge and less exposed thermolabile amino acids (*37–39*). Thus, increased ionic interaction and hydrogen bonds, increased hydrophobicity, decreased flexibility and smaller surface loops confer stability on the thermophilic protein (*37–39*).

To date, a large number of polysaccharide degrading enzymes (*e.g.* cellulases, amylases, pullulanases, xylanases, mannanase, pectinases and chitinases), proteases, lipases, esterases and phytases have been characterized from extremely thermophilic and hyperthermophilic microorganisms. Many of these results have been presented in some excellent reviews (*4–17*). Some recent publications are additionally shown in Table 1a (*40–56*).

Various kinds of thermostable enzymes are required by industries. The detergent, food, feed, starch, textile, leather, pulp and paper and pharmaceutical industries are the major users of enzymes (*4–17,19*). The starch industry is one of the largest users of thermostable amylolytic enzymes (*e.g.* α-amylases, glucoamylases and isoamylases or pullulanases) for the hydrolysis and modification of starch to produce glucose and various other products (*15–17,19*). Amylolytic enzymes are also used in the textile, paper and baking industries. Cellulolytic enzymes are employed in the removal of polyphenolic substances from juices, in detergents for color brightening and softening of textiles, in the bio-stoning of jeans, in the pulp and paper industries and in the pretreatment of plant biomass. Cellulase-free thermostable hemicellulases like xylanases have offered a major step forward in the biobleaching of pulp and paper, thus lowering the environmental pollution by halogens. Nowadays, biodetergents contain enzymes like amylase, protease, cellulase and lipase, using variants that are resistant to harsh conditions. Lipases are also used in various processes, for example, fat hydrolysis, esterification, interesterification, *trans*-esterification and organic biosynthesis. Other applications of lipase include the removal of pitch from pulp produced in the paper industry, the hydrolysis of milk fat in the dairy industry, the removal of non-cellulosic impurities from raw cotton before further processing into dyed and finished products, the removal of subcutaneous fat in the leather industry and manufacturing of drugs in the pharmaceutical industry. Thermostable phytases are added to animal feeds in order to hydrolyze phytic acid (phytate), an antinutritional factor present in cereals and oil seeds, thereby releasing digestible phosphorous (*10,12*). Thus the need to supplement the feed with an external source of phosphorous is reduced. Adding phosphorous to feeds results in excessive excretion of phosphorous in the manure of the animals, which creates an environmental problem. Phytase also releases minerals (including Ca, Mg, Zn and K), amino acids and proteins that are complexed with phytate and therefore improves dietary absorption of minerals. Thermostable xylanases, amylases and proteases are added to animal and poultry feed in order to increase digestibility. Chitinases are used for the hydrolysis of chitin to various useful products.

Thermostable DNA polymerases, isolated from hyperthermophiles, have led to a tremendous advance in molecular biology due to their capacity to amplify DNA, in the so-called polymerase chain reaction (*3,13*). Enzyme-catalyzed synthesis of carbohydrates is now well established. A series of thermophilic glycosidases, sold commercially as CLONEZYMEs™, can be used to catalyze the formation of a range of linkages, including Gal β(1→4), Galβ(1→6), Galα(1→6), D-Fucβ(1→3) and D-Fuc β(1→2) (*57–59*). The synthetic ability of thermophilic enzymes was further confirmed by using β(1→3)-galactosidase from *Thermus thermophilus*, which showed high regioselectivity during the transfer of galactosyl, glucosyl and D-fucosyl residues from *p*-nitrophenyl donors to a variety of saccharide acceptors (*60*). Industrial production of fine chemicals and their respective intermediates as final products, especially in the form of enantiomerically pure compounds, will receive much attention and find increasing applications in biocatalytic industries in the near future.

## Psychrophiles

Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) microorganisms are found inhabiting the low temperature environments of the Earth, including polar regions, high mountains, glaciers, ocean deeps, shallow subterranean systems (*i.e.* caves), the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5 °C. The potentials of psychrophiles and psychrophilic enzymes have been reviewed by Cavicchioli *et al.* (*20*), Deming (*21*), Margesin *et al.* (*22*), Feller and Gerday (*23*) and Georlette *et al.* (*24*). In fact, deep oceans, which cover over 70 % of the Earth's surface, represent the major ecosystem on the planet. Many psychrophiles live in biotopes having more than one stress factors, such as low temperature and high pres-

Table 1a. Production of extremophilic enzymes by hyperthermophiles

| Hyperthermophiles | Thermophilic enzymes | $T_{opt}$/°C | $pH_{opt}$ | Stability | Ref. |
|---|---|---|---|---|---|
| **Bacteria**<br>*Bacillius*, *Clostridia*, *Fervidobacterium pennavorans*, *Rhodothermus marinus*, *Rhodothermus obamensis*, *Thermus caldophilus*, *Thermoanaerobacter* sp., *Thermoplasma acidophylum*, *Thermotoga maritima*, *Thermotoga neapolitana*, *Picrophilus oshimae*, *Picrophilus torridus*<br>**Archaea**<br>*Desulfurococcus mucosus*, *Pyrococcus furiosus*, *Pyrococcus woesei*, *Pyrodictium abyssi*, *Staphylothermus marinus*, *Sulfolobus solfataricus*, *Thermococcus hydrothermalis*, *Thermococcus litoralis*, *Thermococcus celer*, *Thermococcus profundus*, *Thermococcus aggregans* | Cellulase<br>Amylase<br>Pullulanase I<br>Pullulanase II<br>α-Glucosidase<br>β-Glucosidase<br>Glucoamylase<br>Xylanase<br>Mannanase<br>Pectinase<br>Chitinase<br>Protease<br>Lipase<br>Esterase<br>Phytase | | | | *4–17* |
| *Alicyclobacillus acidocaldarius* | Endoglucanase (CelB) | 80 | 4.0 | Stable at pH=1–7, retains 60 % activity after 1 h at 80 °C | *40* |
| Environmental DNA | β-Xylanase | 100 | 6.0 | Stable at 90 °C | *41* |
| *Methanococcus jannaschii* | α-Amylase | 120 | 5.0–8.0 | Stable against denaturants | *42* |
| *Pyrobaculum calidifontis* | Carboxylesterase | 90 | 7.0 | ½ life: 2 h at 100 °C | *43* |
| *Pyrococcus furiosus* | Chitinase a and b | 90–95 | 6.0 | NA | *44* |
| *Pyrodictium abyssi* | Xylanase | 105 | 6.0 | ½ life: 100 min at 105 °C | *45* |
| *Rhodothermus marinus* | Amylase | 85 | 6.5 | ½ life: 3 h at 85 °C | *46* |
| | Pullulanase | 80 | 6.5–7.0 | 30 min at 85 °C | *47* |
| | α-L-Arabinofuranosidase | 85 | 5.5–7.0 | 8.3 h at 85 °C | *48* |
| | β-Mannanase | 85 | 5.0–6.5 | 45.3 h at 85 °C | |
| *Sulfolobus solfataricus* | Xylanase | 100 | 7.0 | ½ life: 47 min at 90 °C | *49* |
| *Sulfolobus solfataricus* | α-Glucosidase | 120 | 4.5 | Highly thermostable (whole cells used) | *50* |
| *Sulfolobus solfataricus* | Trehalosyl transglucoylase | 75 | 5.0 | Stable at pH=4.5–11.0 after 2 h at 80 °C | *51* |
| *Sulfolobus shibatae* | α-Glucosidase | 98 | 5.5 | Retained 67 % activity after 5 h at 80 °C | *52* |
| *Thermococcus litoralis* | L-Aminoacylase | 85 | 8.0 | ½ life: 25 h at 70 °C, 1.7 h at 85 °C | *53* |
| *Ralstonia* sp. A-471 | Chitinase | 70 | 5.0 | NA | *54* |
| *Thermococcus chitonophagus* | Chitinase | 70 | 7.0 | ½ life: 1 h at 120 °C | *55* |
| *Thermoplasma acidophilum* | Glucoamylases | 90 | 2.0 | ½ life: 24 h at 90 °C for *P. torridus* and *T. acidophilum*, 20 h for *P. oshimae* | *56* |
| *Picrophilus torridus* | | 90 | 2.0 | | |
| *Picrophilus oshimae* | | 90 | 2.0 | | |

NA = not available

sure in deep seas (piezo-psychrophiles), or high salt concentration and low temperature in sea ice (halo-psychrophiles) (*20–24*).

A diverse range of psychrophilic microorganisms, belonging to Gram-negative bacteria (*e.g. Pseudoalteromonas*, *Moraxella*, *Psychrobacter*, *Polaromonas*, *Psychroflexus*, *Polaribacter*, *Moritella*, *Vibrio* and *Pseudomonas*), Gram-positive bacteria (*e.g. Arthrobacter*, *Bacillus* and *Micrococcus*), archaea (*e.g. Methanogenium*, *Methanococcoides* and *Halorubrum*), yeast (*Candida* and *Cryptococcus*) and fungi (*Penicillium* and *Cladosporium*) have been isolated from these cold environments (*20–24*). These psychrophiles are able to degrade a wide range of polymeric substances such as starch, cellulose, xylan, pectin, chitin, protein and lipid and produce enzymes like amylase, cellulase, xylanase, pectinases, chitinase, protease and lipase, respectively (*7,8,10,20–24*). In addition, some recent examples are shown in Table 1b (*61–77*).

Psychrophiles have developed adaptive mechanisms to perform their metabolic functions at low temperatures by incorporating unique features in their proteins and membranes (*20–24*). Compared to proteins from mesophiles, psychrophilic proteins show decreased ionic interactions and hydrogen bonds, possess less hydrophobic groups and more charged groups on their surface and longer surface loops (*20–24*). Due to these modifications, at low temperatures psychrophilic proteins lose their rigidity and gain increased structural flexibil-

Table 1b. Production of extremophilic enzymes by psychrophiles/psychrotolerant microorganisms

| Psychrophiles /psychrotolerant | Cold-active enzymes | $T_{opt}$/°C | $pH_{opt}$ | Stability | Ref. |
|---|---|---|---|---|---|
| *Acinetobacter* sp. strain no. 6 | Novel esterase<br>Lipase | 50<br>20 | 7.8<br>7.0 | Lost 75 % activity at 40 °C in 30 min<br>½ life: 30 min at 50 °C | *61* |
| *Arthrobacter* sp. C2-2 | β-Galactosidase | 40 | 7.5 | ½ life: 8 min at 50 °C, stable after 4 h at 40 °C | *62* |
| *Arthrobacter* sp. strain TAD20 | Chitinases | NA | NA | | *63* |
| *Bacillus, Clostridium, Actinomycetes*<br>*Cytophaga-Flexibacter-Bacteroides* | α-Amylase<br>β-Galactosidase | NA | NA | NA | *64* |
| *Pedobacter cryoconitis* sp. nov | Oxidase, catalase, protease, amylase, β-glucosidase, β-galactosidase, β-lactamase | NA | NA | NA | *65* |
| *Clostridium* strain PXYL1 | Filter paper cellulase<br>Endocellulase<br>Xylanase | 20 (for all three) | 5.0–6.0 (for all three) | ½ life: 30 min at 40 °C for xylanase, CMCase and FPase | *66* |
| *Cystofilobasidium larimarini* | Polygalacturonase | 40 | 5.0 | | |
| *Cystofilobasidium capitatum* | Polygalacturonase | 40 | 5.0 | The enzymes were very unstable at 30–40 °C | *67* |
| *Cryptococcus macerans* | Polygalacturonase | 50 | 4.0 | | |
| *Cryptococcus aquaticus* | Polygalacturonase | 50 | 4.0 | | |
| *Cryptococcus adeliae* | Xylanase | 45–50 | 5.0–5.0 | ½ life: 78 min at 35 °C | *68* |
| *Cryptococcus cylindricus*<br>*Mrakia frigida*<br>*Cystofilobasidium capitatum* | Pectinase | NA | NA | | *69* |
| *Flavobacterium psychrophilum* | Metalloprotease | 24 | 6.5 | Lost all activity after 5 min at 40 °C | *70* |
| *Pseudoalteromonas haloplanktis* | Cellulase | NA | NA | NA | *71* |
| *Pseudoalteromonas haloplanktis* | Xylanase (family 8) | 25 | 5.3–8.0 | Mp 52.6 °C<br>½ life: 1.9 min at 55 °C | *72* |
| *Psychrobacter* sp. Ant300 | Esterase | 5–25 | 7.0–9.0 | 16 min at 40 °C | *73* |
| *Psychrobacter okhotskensis* sp. nov | Lipase | NA | NA | NA | *74* |
| *Penicillium chrysogenum* | Endo-arabinanase | 30–40 | 6.0–7.0 | Stable up to 30 °C | *75* |
| *Pseudomonas* strain DY-A | Alkaline protease | 40 | 10.0 | NA | *76* |
| *Bacillus* spp. | Subtilisin | 40–45 | 10.5–11.0 | Thermolabile | *77* |

NA = not available

ity for enhanced catalytic function. As the psychrophilic membranes contain a higher proportion of unsaturated fatty acids, their fluidity and ability to transport nutrients are maintained under very cold conditions. Moreover, the ability to synthesize cold-shock or antifreeze proteins as the temperature drops, the more efficient enzyme activity due to alterations in enzyme kinetics and the stabilization of microtubules enable the psychrophiles to continue their activities (*24*). The adaptive properties of psychrophilic enzymes are their high specific activity at low temperatures, a relatively low apparent temperature optimum for activity and a rather high thermolability.

The ability of psychrophilic enzymes to catalyse reactions at low or moderate temperatures offers great industrial and biotechnological potential (*7,8,10,20–24*). The use of cold-active hydrolytic enzymes such as proteases, lipases, amylases and cellulases in the formulation of detergents would be of great advantage for cold washing. This would reduce the energy consumption and wear and tear of textile fibers. The industrial dehairing of hides and skins at low temperatures using psychrophilic proteases or keratinase would not only save energy but also reduce the impacts of toxic chemicals used in dehairing. Apart from these examples, cold--active enzymes have potential for other interesting applications such as the hydrolysis of lactose in milk using β-galactosidase, biopolishing and stone washing of textile products using cellulases, extraction and clarification of fruit juices using pectinases, tenderization of meat or taste improvement of refrigerated meat using proteases (*78*), improvement of bakery products using glycosidases (*e.g.* amylases, proteases and xylanases), softening of wool or cleaning of contact lenses using proteases. Other cold–active enzymes could be good alternatives to mesophilic enzymes in brewing and wine industries, cheese manufacturing, animal feed supplements, and so on. Psychrophilic microorganisms as well as their enzymes (*e.g.* oxidase, peroxidase, and catalase) have been proposed as alternatives to physicochemical methods for the bioremediation of solids and waste waters polluted by hydrocarbons, oils and lipids (*22*). Psychrophi-

lic enzymes can also be used as environmental biosensors (*e.g.* dehydrogenases) or in biotransformation (*e.g.* methylases, aminotransferases and alanine racemase). Psychrophiles may be a good source of polyunsaturated fatty acids for the pharmaceutical industry. Psychrophiles may be good sources of novel therapeutic agents, for example, many bacteria belonging to the genus *Pseudoalteromonas* possess antibiotic properties (*79*).

## Alkalithermophiles/Alkaliphiles/ Acidothermophiles/Acidophiles

Alkalithermophilic microorganisms grow optimally under two extreme conditions: at pH values of 8 or above and at high temperatures (50–85 °C). Some of the alkalithermophiles require additional salt concentrations for their growth. On the other hand, microorganisms simply classified as alkaliphiles are mesophiles and consist of two main physiological groups: alkaliphiles and haloalkaliphiles (*10,25,26*). Alkaliphiles require an alkaline pH of 8 or more for their growth and have an optimal growth pH of around 10, whereas haloalkaliphiles require both an alkaline pH (pH>8) and high salinity (up to $m/V$ (NaCl) = 33 %). Alkalithermophiles as well as alkaliphiles have been isolated mainly from alkaliphilic and thermobiotic environments such as alkaline hot springs, the new alkaline hydrothermal vents of the »Lost City« and alkaline lakes in Africa, Egypt and Israel. However, various alkalithermophiles have also been isolated from mesobiotic, slightly acidic to neutral environments, sometimes even from acidic soil samples and sewage. Haloalkaliphiles have been mainly found in extremely alkaline saline environments, such as the Rift Valley lakes of East Africa and the western soda lakes of the United States (*25*). Some of the aspects of alkalithermophiles and alkaliphiles have been presented in recent reviews by Wiegel (*25*) and Horikoshi (*26*), respectively.

Acidothermophiles thrive under conditions of low pH and high temperature. For example, the acidothermophile *Sulfolobus solfataricus* grows at pH=3 and 80 °C. True acidophiles such as the archaea *Picrophilus torridus* and *Picrophilus oshimae* grow optimally at pH values as low as 0.7 and at 60 °C and produce starch-hydrolyzing enzymes (amylases, pullulanases, glucoamylases and glucosidases) (*56*).

One of the most striking properties of acidophilic and alkaliphilic microorganisms is their use of proton pumps to maintain a neutral pH internally, and so the intracellular enzymes from these microorganisms do not need to be adapted to extreme growth conditions. However, the extracellular enzyme proteins of acidophiles have to function at low pH whereas those of alkaliphiles function at alkaline pH. How these extracellular proteins operate at high or low pH values is yet poorly un-

Table 1c. Production of extremophilic enzymes by thermoalkaliphiles/alkaliphiles

| **Thermoalkaliphiles / alkaliphiles** | **Thermoalkaliphilic / alkaliphilic enzymes** | $T_{opt}$/°C | $pH_{opt}$ | **Stability** | **Ref.** |
|---|---|---|---|---|---|
| *Alkalimonas amylolytica* | Amylase | NA | NA | NA | *80* |
| *Streptomyces* sp. | Endocellulase | 50 | 8.0 | Retained 95 % activity at opt. temp and pH for 30 min | *82* |
| *Bacillus firmus* | Xylanases (xyn10A and xyn11A) | 70 | 5.0–9.5 | Retained ca. 70 % activity after 16 h at 62 °C | *83* |
| *Bacillus halodurans* strains | Amylase, pullulanase | 55–65 | 10.0 | NA | *84* |
| *Bacillus subtilis* | α-Amylase | 52–55 | 9.0 | Lost 60 % activity after 10 min at 95 °C | *85* |
| *Bacillus* isolate KSM-K38 | α-Amylase | 55–60 | 8.0–9.0 | Highly resistant to chelating reagents and chemical oxidants | *86* |
| *Nesterenkonia* sp. AL-20 | Alkaline protease | *T*m=74 °C | 10.0 | Highly stable against $H_2O_2$ and sequestering agents | *87* |
| *Bacillus pumilus* | Alkaline protease | 50–60 | 11.5 | NA | *88* |
| *Arthrobacter ramosus*<br>*Bacillus alcalophilus* | Alkaline protease | 65 | 11.0<br>10.0 | Both enzymes are stable at 30–65 °C and pH=7–12 | *89* |
| *Nocardiopsis* sp. | Alkaline protease<br>Keratinase | 70–75 | 11.0–11.5 | Stable below 60 °C for 10 min and pH=8 | *90* |
| *Pseudomonas* sp. LBA34<br>*Halomonas* sp. LBB1 | Lipase | NA | NA | NA | *91* |
| *Bacillus* sp. | Azoreductase | 80 | 8.0–9.0 | NA | *92* |
| *Bacillus* sp. | Catalase-peroxidase | 60 | 8.0 | ½ life: 20 h at pH=9 and 60 °C | *93* |
| *Thermus brockianus* | Catalase | 90 | 8.0 | ½ life: 3 h at 90 °C, 330 h at 80 °C | *94* |
| *Bacillus alcalophillus* | Pectate lyase | 45 | 9.0–10.0 | NA | *95* |
| *Thermomonospora* (actinomycete) | Endocellulase | 50 | 5.0 | Stable at pH=7–10<br>½ life: 3 h at 70 °C | *96* |

NA = not available

derstood. In order for the cells to survive in the aggressive conditions of pH, alkaliphiles and acidophiles utilize several strategies. Alkaliphiles have negatively charged cell-wall polymers in addition to peptidoglycan, which may reduce the charge density at the cell surface and help to stabilize the cell membrane (*25,26*). Cellular fatty acids in alkaliphilic bacterial strains contain predominantly saturated and mono-unsaturated straight-chain fatty acids (*25,26,80*). In order to withstand low pH, acidophiles employ a range of mechanisms such as: a positively charged membrane surface (*81*), a high internal buffer capacity, over-expression of $H^+$ exporting enzymes and unique transport systems (*25,26*).

Thermoalkaliphiles and alkaliphiles are good sources of alkaliphilic enzymes like cellulases, xylanases, amylases, proteases, lipases, pectinases, chitinase, catalase, peroxidase and oxidoreductase (*25,26*). Some recent reports on these enzymes are shown in Table 1c (*80, 82–96*). Thermoalkaliphilic enzymes have great biocatalytic potential in processes that are performed at alkaline pH and higher temperatures. For example, proteases, lipases and cellulases are used as additives in laundry and dishwashing detergents, proteases are also used for dehairing of hides and skins and to improve smoothness and dye affinity of wool (*10,25,26,97*). Cellulase-free xylanases are used for biobleaching of pulp and paper, pectinases are used in degumming of ramie fibers and catalase and peroxidase or oxidoreductase may be used to remove residual hydrogen peroxide from effluent streams of the textile processing industry (*25,26,92–94*). Due to their versatile uses, alkaliphilic enzyme-producing bacteria and archaea have received great attention in recent years (*25,26*).

## Halophiles

Halophilic microorganisms require very high salt (NaCl) concentrations for growth. They are found in salterns and hypersaline lakes, such as the Great Salt Lake, the Dead Sea and solar lakes in Africa, Europe and the USA. They have even been found in Antarctic lakes. They accumulate salts such as NaCl or KCl up to concentrations that are isotonic with the environment. As a result, proteins from halophiles have to cope with very high salt concentrations (up to about 4 M KCl and over 5 M NaCl (*5,10,12,27*). The molecular ecology of extremely halophilic archaea and bacteria has been reviewed by Oren (*27*). Halophiles like *Halobacterium*, *Haloferax*, *Haloarcula*, *Halococcus*, *Natronobacterium* and *Natronococcus* belong to the archaea while *Salinibacter ruber* is a bacterium (*27*).

Halophilic proteins employ different adaptation mechanisms. Proteins from halophilic organisms have a biased amino acid composition in order to remain stable and active at high ionic strength. Halophilic proteins typically have an excess of acidic amino acids (*i.e.* glutamate and aspartate) on their surface (*98–101*), although such a high proportion of acidic amino acids is not present in the amylase from the thermophilic halophile *Halothermothrix orenii* (*102*). Negative charges on the halophilic proteins bind significant amounts of hydrated ions, thus reducing their surface hydrophobicity and decreasing the tendency to aggregate at high salt concentration. Halophilic proteins are distinguished from their non-halophilic homologous proteins by exhibiting remarkable instability in solutions with low salt concentrations and by maintaining soluble and active conformations in high concentrations of salt, for example, up to 5 M NaCl (*98–101*).

Halophiles respond to increases in osmotic pressure in different ways. The extremely halophilic archaea, the *Halobacteriaceae*, accumulate $K^+$, while other bacteria accumulate compatible solutes (*e.g.* glycine, betaine, sugars, polyols, amino acids and ectoines), which help them to maintain an environment isotonic with the growth medium (*98,99*). These substances also help to protect cells against stresses like high temperature, desiccation and freezing. Consequently, in surroundings with lower salt concentrations, the solubility of halophilic proteins is often very low (*98–101*).

Halophiles from the archaeal domain provide the main source of extremely halophilic enzymes. The potentials of halophiles and haloenzymes have been reviewed previously (*5,10,14,27,98–100*). The production of halophilic enzymes, such as xylanases, amylases, proteases and lipases, has been reported for some halophiles belonging to the genera *Acinetobacter*, *Haloferax*, *Halobacterium*, *Halorhabdus*, *Marinococcus*, *Micrococcus*, *Natronococcus*, *Bacillus*, *Halobacillus* and *Halothermothrix* (*4,10,14,27,98–101*). Table 1d shows some recent reports on these enzymes (*102–108*).

However, many of these enzymes have not been investigated in detail or applied (*14*). Although the halophilic enzymes can perform enzymatic functions identical to those of their non-halophilic counterparts, these enzymes have been shown to exhibit substantially different properties, among them the requirement for high salt concentrations (1–4 M NaCl) for activity and stability and a high excess of acidic over basic amino residues (*101*). It is argued that the high negative surface charge of halophilic proteins makes them more soluble and renders them more flexible at high salt concentrations, conditions under which non-halophilic proteins tend to aggregate and become rigid. This high surface charge is neutralized mainly by tightly bound water dipoles (*98–101*). The requirement of high salt concentration for the stabilization of halophilic enzymes, on the other hand, is due to a low affinity binding of the salt to specific sites on the surface of the folded polypeptide, thus stabilizing the active conformation of the protein (*101*). The dependence of the stability and the catalytic properties of halobacterial proteins on salt concentration has been the subject of studies for many years. The property of low solubility of halophilic enzymes has been taken advantage of by applying them in aqueous/organic and non-aqueous media (*109,110*). To date, the use of halophilic extremozymes in organic solvents has been limited to very few enzymes (*14*). To explore the behaviour of these extremozymes in organic media, different approaches have been adopted, including the dispersal of the lyophilized enzyme or the use of reverse micelles (*110,111*). In fact, the use of reverse micelles in maintaining high activities of halophilic extremozymes under unfavourable conditions could open new fields of application such as the use of these enzymes as biocatalysts in organic media. The reverse micelles are considered as

Table 1d. Production of extremophilic enzymes by halophiles

| Halophiles | Halophilic enzymes | $T_{opt}$/°C | $pH_{opt}$ | Stability | Ref. |
|---|---|---|---|---|---|
| *Halothermothrix orenii* | α-Amylase | 65 | 7.5 | Tolerates up to 25 % NaCl<br>$T_{opt}$ at 5 % NaCl | *102* |
| *Bacillus dipsosauri* | α-Amylase | 60 | 6.5 | Stable up to 60 °C | *103* |
| *Halobacillus* sp. strain MA-2 | Amylase | 50 | 7.5–8.5 | Maximum stable at 5 % NaCl | *104* |
| *Haloferax mediterranei* | α-Amylase | 50–60 | 7–8 | Stable at 2–4 M NaCl<br>Optimum 3 M NaCl | *105* |
| Halophilic bacterium, CL8 | Xylanase 1<br>Xylanase 2 | 60<br>65 | 6.0<br>6.0 | Stable 7 min at 60 °C<br>Stable 192 min at 60 °C<br>($T_{opt}$ at 4 M NaCl) | *106* |
| *Halorhabdus utahensis* | β-Xylanase<br>β-Xylosidase | 55, 70<br>65 | NA<br>NA | Optimum activity at 5–15 % NaCl<br>Optimum activity at 5 % NaCl | *107* |
| *Pseudoalteromonas* sp. strain CP76 | Protease CPI | 55 | 8.5 | Tolerates 0–4 M NaCl<br>Optimum activity at 7.5 % NaCl | *108* |

NA = not available

micro-reactors, which are very dynamic and change with the environment conditions (*110,111*). Halophilic enzymes encapsulated in reverse micelles can keep their catalytic properties in organic solvents, even at low salt concentrations. For example, the manufacture of chemicals such as pesticides, herbicides, explosives and dyes usually generates effluents containing complex mixtures of xenobiotic compounds, salt and organic solvents. Halophilic extremozymes encapsulated in reverse micelles may be used in bioremediation (*i.e.* to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes) (*110–111*).

## Piezophiles

Microorganisms that like high-pressure conditions for growth are termed piezophiles (formerly known as barophiles). With an average pressure of 38 MPa, the world's oceans are home to piezophiles, including various thermophiles and hyperthermophiles (*28*). Piezophiles are distributed among the genera *Shewanella, Colwellia, Moritella, Methanococcus, Pyrococcus* and *Thermus* (*28,29*).

The biotechnological potentials of piezophiles and piezoenzymes have recently been reviewed by Abe and Horikoshi (*28*) and Yano and Poulos (*29*). Piezophilic enzymes that are stable at high pressures have been isolated from a wide variety of microorganisms, most of which are either thermophilic or psychrophilic and have optimal growth conditions above one atmosphere (*28,29*). The molecular basis of piezophily is now being investigated extensively, focusing on aspects of gene regulation and the function of certain proteins in deep-sea isolates. Recent studies indicate that the important global factors for increased thermal stability are surface electrostatics, amino acid composition (such as a decrease in thermolabile residues), shorter loops, increased charge dipole on helices and interactions between cations and aromatic rings (cation–π interactions) (*29*).

Based on the facts that pressures exceeding 400 MPa are needed to induce protein denaturation, and microorganisms living in the deep sea are not exposed to pressures exceeding 120 MPa (*112*) it is assumed that pressure does not represent a major selective factor for protein structure and function in piezophiles and that their proteins do not need specific pressure-related adaptations (*8,29*). However, there are some examples of protein stabilization by high pressure (*113,114*).

During processing and sterilization of food materials, high pressures of a few hundred MPa can be used to induce the formation of gels or starch granules, the denaturation/coagulation of proteins or the transition of lipid phases. The use of high pressure leads to better flavor and color preservation than the use of high temperature to achieve the same ends (*28,29,115,116*). Moreover, enzymes that can operate at increased pressure and temperature have great advantages in biotechnological applications. Enzymatic reactions that have a negative change in activation volume ($\Delta V<0$) are favored by increasing pressure, whereas reactions with a positive change ($\Delta V>0$) are not. The change in activation volume ($\Delta V$) can be used as a method to control reaction specificity (*29*). For example, α-chymotrypsin catalyzes both the hydrolysis of an anilide ($\Delta V<0$) and the hydrolysis of an ester ($\Delta V>0$). A reaction mixture containing both substrates and α-chymotrypsin in organic media was controlled by altering the pressure to favor one or the other reaction (*117*).

Although there are many possible biotechnological applications of piezophiles and piezophilic enzymes, there are few known practical applications of piezophiles or piezophilic enzymes (*28,29*). This is due to the fact that it is not easy to cultivate piezophiles under high-pressure conditions using current technology. Therefore, the properties of these enzymes and other cellular components have not yet been fully investigated.

## Radiophiles

Microorganisms that are highly resistant to high levels of ionizing and ultraviolet radiation are called radiophiles. The genetic engineering and environmental biotechnology aspects of radiophiles have been re-

viewed by Daly (*118*). Examples of radiophiles are *Deinococcus radiodurans* (*119*), *Deinococcus radiophilus* (*120*), *Thermococcus marinus* sp. nov. and *Thermococcus radiotolerans* (*121*). *Deinococcus radiodurans* is a remarkable bacterium that is highly resistant to chemicals, oxidative damage, high levels of radiation (5 Mrad, 3000 times higher than what would kill a human) and dehydration. It was first isolated in 1956 from a radiation »sterilized« can of ground meat. It contains a spectrum of genes that encode for multiple activities that repair DNA damage. The genes of three putative uracil-DNA glycosylases have been cloned and expressed to determine their biochemical function (*119*). DR0689 is a homologue of the *E. coli* uracil-DNA glycosylase, the product of the »ung« gene. Unlike its thermophilic relatives, the enzyme is not heat stable. The purified enzyme superoxide dismutase of another member of the genus, *Deinococcus radiophilus*, is stable at pH=5.0–11.0, but quite unstable below pH=5.0. It is thermostable up to 40 °C, but a linear reduction in activity occurs above 50 °C (*120*).

The presence of toxic chemicals, heavy metals, halogenated solvents and radionuclides in many nuclear waste materials presents a challenging problem for separating different species and disposing of individual contaminants. *Deinococcus radiodurans* strains and other detoxifying microorganisms may be utilized to detoxify halogenated organics and toxic metals such as mercury, and theoretically could be used to remove these classes of compounds selectively from mixed wastes under mild conditions. Several research groups are using genetic engineering techniques to develop suites of radiation resistant bacterial strains to do this (*118*).

## Metallophiles

Microorganisms that can grow in the presence of high metal concentrations are called metallophiles. These organisms, including several members of the genus *Ralstonia*, colonize industrial sediments, soils or wastes with high contents of heavy metals. Metal-resistant *Ralstonia* have adapted well to the harsh environments created by extreme anthropogenic activities or biotopes (*122*). *Ralstonia metallidurans*, a gram-negative, non-spore forming *Bacillus*, thrives in millimolar concentrations of toxic heavy metals. It was first isolated in 1976 from the sludge of a zinc decantation tank in Belgium that was polluted with high concentrations of several heavy metals. A typical feature of these metal-resistant *Ralstonia* is the presence of one or two large megaplasmids that contain genes for multiple resistances to heavy metals. These plasmids confer resistance to Zn, Cd, Co, Pb, Cu, Hg, Ni and Cr.

Since pollution by heavy metals poses a threat to public health, fishery and wildlife, there has been an increased interest in developing systems that can remove or neutralize the toxic effects of heavy metals in soils, sediments and wastewaters (*123*). Many microorganisms, including *Ralstonia*, could be used in heavy metal bioremediation. In addition to their use as biosorbents, bacteria can be used to immobilize certain heavy metals efficiently, this being mediated by their capacity to reduce these elements to a lower redox state, producing metal species that have a lower bioactivity. Bacteria exhibit a number of enzymatic activities that transform certain metal species through oxidation, reduction, methylation and alkylation. Apart from the enzymatic transformations that lead to metal precipitation and immobilisation, other biological reactions that generate less poisonous metal species have been applied to bioremediation (*122,123*).

## Xerophiles

The driest regions in the world can support life, even dry stones and deserts. However, only specialized microbes belonging to fungi, lichens and algae have the ability to grow in such extremely dry conditions. These specialized organisms are called xerophiles (*2,3*). Various xerophiles are responsible for spoiling of dried foods and stored grains, spices, nuts and oilseeds. Since xerophiles live in extremely dry environments, they face the problem of reproducing and creating enzymes that will hydrolyze substrates in conditions of extremely low water contents. Some xerophiles (osmophiles) spoil salty foods, and others spoil sugary foods. As very limited studies have been conducted on xerophiles, the biocatalytic potential of these microbes and their enzymes is not yet known.

## Production of Biomass and Extremophilic Enzymes

The major drawback of extremophilic biomass and enzyme production is the difficulty of cultivating them in laboratory and industrial environments. Difficulties and new developments in the production of biocatalysts from extremophiles have been presented by Schiraldi and De Rosa (*18*). The conditions required for the growth of extremophiles include extreme temperatures, often in an anaerobic environment, and media of extreme pH (for acidophiles and alkaliphiles) or containing up to 5 M NaCl (for halophiles). These conditions are incompatible with standard industrial fermentation and downstream processing equipment. Fermentation at high temperature using corrosive media requires the use of specially designed equipment. The lowering of gas solubility and the instability of substrates and reagents with increase in temperature are other factors of concern. Additional problems that hinder the use of these microbes and their enzymes or metabolites are low specific growth rates and the appearance of product inhibition, even at a very low concentration. Much work is still going on into developing methods by which thermophiles, extreme thermophiles and hyperthermophiles can be effectively cultivated for increased production of extremophilic biomasses, enzymes and biomolecules. To improve biomass production, different research groups have adopted different techniques such as: optimization of the medium composition (*46–48,68*), use of special fermentors (*e.g.* gas-lift fermentor, membrane bioreactors) (*18,124,125*) and use of different modes of fermentation, for example, fed-batch, cell-recycling or continuous cultivation (*18*). Due to the difficulties associated with large-scale culture of extremophiles and downstream processing of extremozymes, corresponding genes from extremophiles have been and are being expressed successfully in mesophilic

hosts such as *Escherichia coli* and yeasts, although difficulties arise when the expressed enzyme requires specific cofactors or metal ions that the host does not utilise (*18*). Most of the extremozymes that have found their way into industrial applications are produced using *E. coli* expression systems (*7*,*10*,*18*).

## Conclusion and Perspectives

The study of extremophiles and extremozymes has added greatly to our understanding of protein folding, stability, structure and function. Extremozymes have great economic potential in many industrial processes (*e.g.* agriculture, food, feed and drinks, detergents, textile, leather, pulp and paper). Although there are controversial opinions about the potential of extremophiles, some companies (*e.g.* Diversa, Genencor International Inc., Novozymes) and several research groups are investing money and time searching for these microbes and novel applications of extremozymes and strongly believe that new discoveries will revolutionize biotechnology. This renewed confidence in enzyme biotechnology may have emerged as a result of the success of genome-based technologies that are currently in use. It is expected that many extremozymes will be discovered in the years to come and the extremozymes will be used in novel biocatalytic processes.

Since the cultivation of extremophiles is associated with many potential difficulties, the authors believe that only genetic engineering of the desired extremozymes into mesophilic hosts will allow large-scale production of these extremozymes. Our experience with extremophiles leads us to caution that it will take a lot of research to turn extremozymes into industrial products. We strongly believe that discoveries of new extremophiles and genetic engineering of the newly isolated as well as of the currently available extreme microbes will offer novel opportunities for biocatalysis and biotransformations.

## References


1. R. D. MacElroy, Some comments on the evolution of extremophiles, *Biosystems*, *6* (1974) 74–75.
2. M. T. Madigan, B. L. Marrs, Extremophiles, *Sci. Am. 276* (1997) 66–71.
3. L. J. Rothschild, R. L. Manicinelli, Life in extreme environments, *Nature, 409* (2001) 1092–1101.
4. M. W. W. Adams, F. B. Perler, R. M. Kelly, Extremozymes: Expanding the limits of biocatalysis, *Bio/technology*, *13* (1995) 662–668.
5. D. W. Hough, M. J. Danson, Extremozymes, *Curr. Opin. Chem. Biol. 3* (1999) 39–46.
6. F. Niehaus, C. Bertoldo, M. Kähler, G. Antranikian, Extremophiles as a source of novel enzymes for industrial application, *Appl. Microbiol. Biotechnol. 51* (1999) 711–729.
7. D. C. Demirjian, F. Moris-Varas, C. S. Cassidy, Enzymes from extremophiles, *Curr. Opin. Chem. Biol. 5* (2001) 144–151.
8. B. Van den Burg, Extremophiles as a source for novel enzymes, *Curr. Opin. Microbiol. 6* (2003) 213–218.
9. J. A. Irwin, A. W. Baird, Extremophiles and their application to veterinary medicine, *Irish Vet. J. 57* (2004) 348–354.
10. J. Eichler, Biotechnological uses of archaeal extremozymes, *Biotechnol. Adv. 19* (2001) 261–278.
11. C. Vieille, G. J. Zeikus, Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability, *Microbiol. Mol. Biol. Rev. 65* (2001) 1–43.
12. G. D. Haki, S. K. Rakshit, Developments in industrially important thermostable enzymes: a review, *Bioresour. Technol. 89* (2003) 17–34.
13. S. Fujiwara, Extremophiles: Developments of their special functions and potential resources, *J. Biosci. Bioeng. 94* (2002) 518–525.
14. G. A. Sellek, J. B. Chaudhuri, Biocatalysis in organic media using enzymes from extremophiles, *Enzyme Microb. Technol. 25* (1999) 471–482.
15. E. Lévêque, S. Janeek, B. Haye, A. Belarbi, Thermophilic archaeal amylolytic enzymes, *Enzyme Microb. Technol. 26* (2000) 3–14.
16. C. Bertoldo, G. Antranikian, Starch-hydrolyzing enzymes from thermophilic archaea and bacteria, *Curr. Opin. Chem. Biol. 6* (2002) 151–160.
17. M. J. E. C. Van der Maarel, B. Van der Veen, J. C. M. Uitdehaag, H. Leemhuis, L. Dijkhuizen, Properties and application of starch-converting enzymes of the α-amylase family, *J. Biotechnol. 94* (2002) 137–155.
18. C. Schiraldi, M. De Rosa, The production of biocatalysts and biomolecules from extremophiles, *Trends Biotechnol. 20* (2002) 515–521.
19. J. R. Cherry, A. L. Fidantsef, Directed evolution of industrial enzymes: an update, *Curr. Opin. Biotechnol. 14* (2003) 438–443.
20. R. Cavicchioli, K. S. Siddiqui, D. Andrews, K. R. Sowers, Low-temperature extremophiles and their applications, *Curr. Opin. Biotechnol. 13* (2002) 253–261.
21. J. W. Deming, Psychrophiles and polar regions, *Curr. Opin. Microbiol. 5* (2002) 301–309.
22. R. Margesin, G. Feller, C. Gerday, N. Russell: Cold-Adapted Microorganisms: Adaptation Strategies and Biotechnological Potential. In: *The Encyclopedia of Environmental Microbiology*, *Vol. 2*, G. Bitton (Ed.), John Wiley & Sons, New York (2002) pp. 871–885.
23. G. Feller, C. Gerday, Psychrophilic enzymes: hot topics in cold adaptation, *Nat. Rev. Microbiol. 1* (2003) 200–208.
24. D. Georlette, V. Blaise, T. Collins, S. D'Amico, E. Gratia, A. Hoyoux, J.-C. Marx, G. Sonan, G. Feller, C. Gerday, Some like it cold: biocatalysis at low temperatures, *FEMS Microbiol. Rev. 28* (2004) 25–42.
25. J. Wiegel, V. V. Kevbrin, Alkalithermophiles, *Biochem. Soc. Trans. 32* (2004) 193–198.
26. K. Horikoshi, Alkaliphiles: Some applications of their products for biotechnology, *Microbiol. Mol. Biol. Rev. 63* (1999) 735–750.
27. A. Oren, Molecular ecology of extremely halophilic Archaea and Bacteria, *FEMS Microbiol. Ecol. 39* (2002) 1–7.
28. F. Abe, K. Horikoshi, The biotechnological potential of piezophiles, *Trends Biotechnol. 19* (2001) 102–108.
29. J. K. Yano, T. L. Poulos, New understandings of thermostable and piezostable enzymes, *Curr. Opin. Biotechnol. 14* (2003) 360–365.
30. S. M. Barns, C. F. Delwiche, J. D. Palmer, N. R. Pace, Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences, *Proc. Natl. Acad. Sci. USA 93* (1996) 9188–9193.
31. H. Huber, M. J. Hohn, R. Rachel, T. Fuchs, V. C. Wimmer, K. O. Stetter, A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont, *Nature*, *417* (2002) 63–67.
32. H. Huber, M. J. Hohn, K. O. Stetter, R. Rachel, The phylum Nanoarchaeota: Present knowledge and future perspectives of a unique form of life, *Res. Microbiol. 154* (2003) 165–171.

*33.* K. Kashefi, D. R. Lovely, Extending the upper limit for life, *Science*, *301* (2003) 934.

*34.* E. Blöchl, R. Rachel, S. Burggraf, D. Hafenbradl, H. W. Jannasch, K. O. Stetter, *Pyrolobus fumarii*, sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 °C, *Extremophiles*, *1* (1997) 14– 21.

*35.* S. Fujiwara, S. Okuyama, T. Imanaka, The world of archaea: genome analysis, evolution and thermostable enzymes, *Gene*, *179* (1996) 165–170.

*36.* S. Fujiwara, M. Takagi, T. Imanaka: Archaeon *Pyrococcus kodakaraensis* KOD1: Application and Evolution. In: *Biotechnology Annual Review, Vol. 4*, Elsevier (1998) 259–284.

*37.* R. Sterner, W. Liebl, Thermophilic adaptation of proteins, *Crit. Rev. Biochem. Mol. Biol. 36* (2001) 39–106.

*38.* S. Kumar, R. Nussinov, How do thermophilic proteins deal with heat? *Cell. Mol. Life Sci. 58* (2001) 1216–1233.

*39.* A. Paiardini, G. Gianese, F. Bossa, S. Pascarella, Structural plasticity of thermophilic serine hydroxymethyltransferases, *Proteins, 50* (2002) 122–134.

*40.* K. Eckert, E. Schneider, A thermoacidophilic endoglucanase (CelB) from *Alicyclobacillus acidocaldarius* displays high sequence similarity to arabinofuranosidases belonging to family 51 of glycoside hydrolases, *Eur. J. Biochem. 270* (2003) 3593–602.

*41.* A. Sunna, P. L. Bergquist, A gene encoding a novel extremely thermostable 1,4-β-xylanase isolated directly from an environmental DNA sample, *Extremophiles*, *7* (2003) 63–70.

*42.* J. W. Kim, L. O. Flowers, M. Whiteley, T. L. Peeples, Biochemical confirmation and characterization of the family-57-like α-amylase of *Methanococcus jannaschii*, *Folia Microbiol. 46* (2001) 467–73.

*43.* Y. Hotta, S. Ezaki, H. Atomi, T. Imanaka, Extremely stable and versatile carboxylesterase from a hyperthermophilic Archaeon, *Appl. Environ. Microbiol. 68* (2002) 3925–3931.

*44.* J. Gao, M. W. Bauer, K. R. Shockley, M. A. Pysz, R. M. Kelly, Growth of hyperthermophilic archaeon *Pyrococcus furiosus* on chitin involves two family 18 chitinases, *Appl. Environ. Microbiol. 69* (2003) 3119–3128.

*45.* C. M. Andrade, W. B. Aguiar, G. Antranikian, Physiological aspects involved in production of xylanolytic enzymes by deep-sea hyperthermophilic archaeon *Pyrodictium abyssi*, *Appl. Biochem. Biotechnol. 91–93* (2001) 655–669.

*46.* I. Gomes, J. Gomes, W. Steiner, Highly thermostable amylase and pullulanase of the extreme thermophilic eubacterium *Rhodothermus marinus*: production and partial characterization, *Bioresour. Technol. 90* (2003) 207–214.

*47.* J. Gomes, I. Gomes, K. Terler, N. Gubala, G. Ditzelmuller, W. Steiner, Optimisation of culture medium and conditions for α-L-arabinofuranosidase production by the extreme thermophilic eubacterium *Rhodothermus marinus*, *Enzyme Microb. Technol. 27* (2000) 414–422.

*48.* J. Gomes, W. Steiner, Production of a high activity of an extremely thermostable β-mannanase by the thermophilic eubacterium *Rhodothermus marinus*, *Biotechnol. Lett. 20* (1998) 729–733.

*49.* R. Cannio, N. Di Prizito, M. Rossi, A. Morana, A xylan-degrading strain of *Sulfolobus solfataricus:* isolation and characterization of the xylanase activity, *Extremophiles*, *8* (2004) 117–124.

*50.* M. Giuliano, C. Schiraldi, M. R. Marotta, J. Hugenholtz, M. De Rosa, Expression of *Sulfolobus solfataricus* α-glucosidase in *Lactococcus lactis*, *Appl. Microbiol. Biotechnol. 64* (2004) 829–832.

*51.* T. Y. Fang, X. G. Hung, T. Y. Shih, W. C. Tseng, Characterization of the trehalosyl dextrin-forming enzyme from the thermophilic archaeon *Sulfolobus solfataricus* ATCC 35092, *Extremophiles*, *8* (2004) 335–343.

*52.* S. Woosowska, J. Synowiecki, Thermostable β-glucosidase with a broad substrate specificity suitable for processing of lactose-containing products, *Food Chem. 85* (2004) 181–187.

*53.* I. N. Taylor, R. C. Brown, M. Bycroft, G. King, J. A. Littlechild, M. C. Lloyd, C. Praquin, H. S. Toogood, S. J. Taylor, Application of thermophilic enzymes in commercial biotransformation processes, *Biochem. Soc. Trans. 32* (2004) 290–292.

*54.* A. Sutrisno, M. Ueda, Y. Abe, M. Nakazawa, K. Miyatake, A chitinase with high activity toward partially N-acetylated chitosan from a new, moderately thermophilic, chitin-degrading bacterium, *Ralstonia* sp. A-471, *Appl. Microbiol. Biotechnol. 63* (2004) 398–406.

*55.* E. Andronopoulou, C. E. Vorgias, Purification and characterization of a new hyperthermostable, allosamidin-insensitive and denaturation-resistant chitinase from the hyperthermophilic archaeon *Thermococcus chitonophagus*, *Extremophiles*, *7* (2003) 43–53.

*56.* E. E. Serour, G. Antranikian, Novel thermoactive glucoamylases from the thermoacidophilic Archaea *Thermoplasma acidophilum*, *Picrophilus torridus* and *Picrophilus oshimae*, *Antonie Van Leeuwenhoek, 81* (2002) 73–83.

*57.* J. Fang, W. Xie, P. G. Wang, Chemical and enzymatic synthesis of glycoconjugates 3: synthesis of lactosamine by thermophilic galactosidase catalyzed galactosylation on a mulitgram scale, *Tetrahedron Lett. 39* (1998) 919–922.

*58.* J. Li, D. E. Robertson, J. M. Short, P. G. Wang, Chemical and enzymatic synthesis of glycoconjugates 4: control of regioselectivity in high yielding synthesis of (β-D-fucopyranosyl)-O-D-xylopyranosyl disaccharides using a CLONEZYME™ thermophilic glycosidase, *Tetrahedron Lett. 38* (1998) 8963–8966.

*59.* J. Li, D. E. Robertson, J. M. Short, P. G. Wang, Chemical and enzymatic synthesis of glycoconjugates 5: one-pot regioselective synthesis of bioactive galactobiosides using a CLONEZYME™ thermophilic glycosidase library, *Bioorg. Med. Chem. Lett. 9* (1999) 35–38.

*60.* V. Chiffoleau-Giraud, P. Spangenberg, M. Dion, C. Rabiller, Transferase activity of a β-glycosidase from *Thermus thermophilus*: specificities and limits — application to the synthesis of β-[1→3]-disaccharides, *Eur. J. Org. Chem.* (4) (1999) 757–763.

*61.* T. Suzuki, T. Nakayama, T. Kurihara, T. Nishino, N. Esaki, Cold-active lipolytic activity of psychrotrophic *Acinetobacter* sp. strain no. 6, *J. Biosci. Bioeng. 92* (2001) 144–148.

*62.* P. Karasová-Lipovová, H. Strnad, V. Spiwok, S. Malá, B. Králová, N. J. Russell, The cloning, purification and characterisation of a cold-active β-galactosidase from the psychrotolerant Antarctic bacterium *Arthrobacter* sp. C2-2, *Enzyme Microb. Technol. 33* (2003) 836–844.

*63.* K. Mavromatis, M. Lorito, S. L. Woo, V. Bouriotis, Mode of action and antifungal properties of two cold-adapted chitinases, *Extremophiles*, *7* (2003) 385–390.

*64.* T. Groudieva, M. Kambourova, H. Yusef, M. Royter, R. Grote, H. Trinks, G. Antranikian, Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen, *Extremophiles* (2004) in press.

*65.* R. Margesin, C. Sproer, P. Schumann, F. Schinner, *Pedobacter cryoconitis* sp. nov., a facultative psychrophile from alpine glacier cryoconite, *Int. J. Syst. Evol. Microbiol. 53* (2003) 1291–1296.

*66.* G. Akila, T. S. Chandra, A novel cold-tolerant *Clostridium* strain PXYL1 isolated from a psychrophilic cattle manure digester that secretes thermolabile xylanase and cellulase, *FEMS Microbiol. Lett. 219* (2003) 63–67.

*67.* H. Birgisson, O. Delgado, L. G. Arroyo, R. Hatti-Kaul, B. Mattiasson, Cold-adapted yeasts as producers of cold-active polygalacturonases, *Extremophiles*, *7* (2003) 185–193.

*68.* J. Gomes, I. Gomes, W. Steiner, Thermolabile xylanase of the Antarctic yeast *Cryptococcus adeliae*: production and properties, *Extremophiles, 4* (2000) 227–235.

*69.* T. Nakagawa, T. Nagaoka, S. Taniguchi, T. Miyaji, N. Tomizuka, Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes, *Lett. Appl. Microbiol. 38* (2004) 383–387.

*70.* P. Secades, B. Alvarez, J. A. Guijarro, Purification and properties of a new psychrophilic metalloprotease (Fpp2) in the fish pathogen *Flavobacterium psychrophilum*, *FEMS Microbiol. Lett. 226* (2003) 273–279.

*71.* S. Violot, R. Haser, G. Sonan, D. Georlette, G. Feller, N. Aghajari, Expression, purification, crystallization and preliminary X-ray crystallographic studies of a psychrophilic cellulase from *Pseudoalteromonas haloplanktis*, *Acta Crystallogr. Sect. D-Biol. Crystallogr. 59* (2003) 1256–1258.

*72.* T. Collins, M. A. Meuwis, I. Stals, M. Claeyssens, G. Feller, C. Gerday, A novel family 8 xylanase, functional and physicochemical characterization, *J. Biol. Chem. 277* (2002) 35133–35139.

*73.* L. Kulakova, A. Galkin, T. Nakayama, T. Nishino, N. Esaki, Cold-active esterase from *Psychrobacter* sp. Ant300: gene cloning, characterization, and the effects of Gly→Pro substitution near the active site on its catalytic activity and stability, *BBA – Proteins Proteomics, 1696* (2004) 59–65.

*74.* I. Yumoto, K. Hirota, Y. Sogabe, Y. Nodasaka, Y. Yokota, T. Hoshino, *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea, *Int J. Syst. Evol. Microbiol. 53* (2003) 1985–1989.

*75.* T. Sakamoto, H. Ihara, S. Kozakic, H. Kawasaki, A cold-adapted endo-arabinanase from *Penicillium chrysogenum*, *Biochim. Biophys. Acta-Gen. Subj. 1624* (2003) 70–75.

*76.* R. Zeng, R. Zhang, J. Zhao, N. Lin, Cold-active serine alkaline protease from the psychrophilic bacterium *Pseudomonas strain* DY-A: enzyme purification and characterization, *Extremophiles, 7* (2003) 335–337.

*77.* M. Okuda, N. Sumitomo, Y. Takimura, A. Ogawa, K. Saeki, S. Kawai, T. Kobayashi, S. Ito, A new subtilisin family: nucleotide and deduced amino acid sequences of new high-molecular-mass alkaline proteases from *Bacillus* spp., *Extremophiles, 8* (2004) 229–235.

*78.* H. He, X. Chen, J. Li, Y. Zhang, P. Gao, Taste improvement of refrigerated meat treated with cold-adapted protease, *Food Chem. 84* (2004) 307–311.

*79.* O. M. Onyshchenko O. A. Kiprianova, T. H. Lysenko, V. V. Smirnov, Antibiotic properties of the *Pseudoalteromonas* genus bacteria isolated from the Black Sea water and mollusks, *Mikrobiol Z. 64* (2002) 38–44.

*80.* Y. Ma, Y. Xue, W. D. Grant, N. C. Collins, A. W. Duckworth, R. P. van Steenbergen, B. E. Jones, *Alkalimonas amylolytica* gen. nov., sp. nov., and *Alkalimonas delamerensis* gen. nov., sp. nov., novel alkaliphilic bacteria from soda lakes in China and East Africa, *Extremophiles, 8* (2004) 193–200.

*81.* N. S. Kar, A. K. Dasgupta, The possible role of surface charge in membrane organization in an acidophile, *Indian J. Biochem. Biophys. 33* (1996) 398–402.

*82.* P. Van Solingen, D. Meijer, W. A. van der Kleij, C. Barnett, R. Bolle, S. D. Power, B. E. Jones, Cloning and expression of an endocellulase gene from a novel streptomycete isolated from an East African soda lake, *Extremophiles, 5* (2001) 333–341.

*83.* P. Chang, W. S. Tsai, C. L. Tsai, M. J. Tseng, Cloning and characterization of two thermostable xylanases from an alkaliphilic *Bacillus firmus*, *Biochem. Biophys. Res. Commun. 319* (2004) 1017–1025.

*84.* S. O. Hashim, O. Delgado, R. Hatti-Kaul, F. J. Mulaa, B. Mattiasson, Starch hydrolysing *Bacillus halodurans* isolates from a Kenyan soda lake, *Biotechnol. Lett. 26* (2004) 823–828.

*85.* K. Das, R. Doley, A. K. Mukherjee, Purification and biochemical characterization of a thermostable, alkaliphilic, extracellular α-amylase from *Bacillus subtilis* DM-03, isolated from the traditional fermented food of India, *Biotechnol. Appl. Biochem. 40* (2004) 291–298.

*86.* H. Hagihara, K. Igarashi, Y. Hayashi, K. Endo, K. Ikawa-Kitayama, K. Ozaki, S. Kawai, S. Ito, Novel α-amylase that is highly resistant to chelating reagents and chemical oxidants from the alkaliphilic *Bacillus* isolate KSM-K38, *Appl. Environ. Microbiol. 67* (2001) 1744–1750.

*87.* S. Bakhtiar, M. M. Andersson, A. Gessesse, B. Mattiasson, R. Hatti-Kaul, Stability characteristics of a calcium-independent alkaline protease from *Nesterenkonia* sp., *Enzyme Microb. Technol. 32* (2003) 525–531.

*88.* C. G. Kumar, Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*, *Lett. Appl. Microbiol. 34* (2002) 13–17.

*89.* P. P. Kanekar, S. S. Nilegaonkar, S. S. Sarnaik, A. S. Kelkar, Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India, *Bioresour. Technol. 85* (2002) 87–93.

*90.* S. Mitsuiki, M. Sakai, Y. Moriyama, M. Goto, K. Furukawa, Purification and some properties of a keratinolytic enzyme from an alkaliphilic *Nocardiopsis* sp. TOA-1, *Biosci. Biotechnol. Biochem. 66* (2002) 164–167.

*91.* V. A. Vargas, O. D. Delgado, R. Hatti-Kaul, B. Mattiasson, Lipase-producing microorganisms from a Kenyan alkaline soda lake, *Biotechnol. Lett. 26* (2004) 81–86.

*92.* J. Maier, A. Kandelbauer, A. Erlacher, A. Cavaco-Paulo, G. M. Gubitz, A new alkali-thermostable azoreductase from *Bacillus* sp. strain SF, *Appl. Environ. Microbiol. 70* (2004) 837–844.

*93.* A. Paar, A. Raninger, F. de Sousa, I. Beurer, A. Cavaco-Paulo, G. M. Gübitz, Production of catalase-peroxidase and continuous degradation of hydrogen peroxide by an immobilized alkalothermophilic *Bacillus* sp., *Food Technol. Biotechnol. 41* (2003) 101–104.

*94.* V. S. Thompson, K. D. Schaller, W. A. Apel, Purification and characterization of a novel thermo-alkali-stable catalase from *Thermus brockianus*, *Biotechnol Progr. 19* (2003) 1292–1299.

*95.* C. Zhai, J. Cao, Y. Wang, Cloning and expression of a pectate lyase gene from *Bacillus alcalophillus* NTT33, *Enzyme Microb. Technol. 33* (2003) 173–178.

*96.* S. P. George, A. Ahmad, M. B. Rao, Studies on carboxymethyl cellulase produced by an alkalothermophilic actinomycete, *Bioresour. Technol. 77* (2001) 171–175.

*97.* K. Schumacher, E. Heine, H. Hocker, Extremozymes in wool finishing – correlation of labor/industry criteria, *DWI Reports, 125* (2002) 376–381.

*98.* M. J. Danson, D. W. Hough, The structural basis of protein halophilicity, *Comp. Biochem. Physiol. 117* (1997) 307–312.

*99.* M. S. Da Costa, H. Santos, E. A. Galinski, An overview of the role and diversity of compatible solutes, *Adv. Biochem. Engin. Biotechnol. 61* (1998) 117–153.

*100.* D. Madern, C. Ebel, G. Zaccai, Halophilic adaptation of enzymes, *Extremophiles, 4* (2000) 91–98.

*101.* M. Mevarech, F. Frolow, L. M. Gloss, Halophilic enzymes: proteins with a grain of salt, *Biophys. Chem. 86* (2000) 155–164.

*102.* B. N. Mijts, B. K. Patel, Cloning, sequencing and expression of an α-amylase gene, amyA, from the thermophilic halophile *Halothermothrix orenii* and purification and bio-

chemical characterization of the recombinant enzyme, *Microbiology*, *148* (2002) 2343–2349.

*103.* C. E. Deutch, Characterization of a salt-tolerant extracellular α-amylase from *Bacillus dipsosauri*, *Lett. Microbiol. 35* (2002) 78–84.

*104.* M. A. Amoozegar, F. Malekzadeh, A. M. Khursheed, Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain MA-2, *J. Microbiol. Methods, 52* (2003) 353–359.

*105.* F. Perez-Pomares, V. Bautista, J. Ferrer, C. Pire, F. C. Marhuenda-Egea, M. J. Bonete, Alpha-amylase activity from the halophilic archaeon *Haloferax mediterranei*, *Extremophiles*, *7* (2003) 299–306.

*106.* P. L. Wejse, K. Ingvorsen, K. K. Mortensen, Purification and characterisation of two extremely halotolerant xylanases from a novel halophilic bacterium, *Extremophiles*, *7* (2003) 423–431.

*107.* M. Waino, K. Ingvorsen, Production of β-xylanase and β-xylosidase by the extremely halophilic archaeon *Halorhabdus utahensis*, *Extremophiles*, *7* (2003) 87–93.

*108.* C. Sanchez-Porro, E. Mellado, C. Bertoldo, G. Antranikian, A. Ventosa, Screening and characterization of the protease CP1 produced by the moderately halophilic bacterium *Pseudoalteromonas* sp. strain CP76, *Extremophiles, 7* (2003) 221–228.

*109.* A. M. Klibanov, Improving enzymes by using them in organic solvents, *Nature, 409* (2001) 241–246.

*110.* F. C. Marhuenda-Egea, M. J. Bonete, Extreme halophilic enzymes in organic solvents, *Curr. Opin. Biotechnol. 13* (2002) 385–389.

*111.* F. C. Marhuenda-Egea, S. Piere-Velazquez, C. Cadenas, E. Cadenas, An extreme halophilic enzyme active at low salt in reversed micelles, *J. Biotechnol. 93* (2002) 159–164.

*112.* M. Gros, R. Jainicke, Protein under pressure, *Eur. J. Biochem. 221* (1994) 617–630.

*113.* R. J. Pledger, B. Crump, J. A. Baros, A barophilic response by two hyperthermophilic, hydrothermal vent Archaea: an upward shift in the optimal temperature and acceleration of growth rate at supra-optimal temperatures by elevated pressure, *FEMS Microbiol. Ecol. 14* (1994) 233–242.

*114.* D. J. Hei, D. S. Clark, Pressure stabilization of proteins from extreme thermophiles, *Appl. Environ. Microbiol. 60* (1994) 932–999.

*115.* R. Hayashi: Use of High Pressure in Bioscience and Biotechnology. In: *High Pressure Bioscience and Biotechnology*, R. Hayashi, C. Balny (Eds.), Elsevier (1996) pp. 1–6.

*116.* H. Ludwig, W. Scigalla, B. Sojka: Pressure and Temperature Induced Inactivation of Microorganisms. In: *High Pressure Effects in Molecular Biophysics and Enzymology*, J. L. Markley, D. B. Northrop, C. A. Royer (Eds.), Oxford University Press, New York (1996) pp. 346–363.

*117.* V. V. Mozhaev, N. Bec, C. Balny, Pressure effects on enzyme reactions in mainly organic media: α-chymotrypsin in reversed micelles of Aerosol OT in octane, *Biochem. Mol. Biol. Int. 34* (1994) 191–199.

*118.* M. J. Daly, Engineering radiation-resistant bacteria for environmental biotechnology, *Curr. Opin. Biotechnol. 11* (2000) 280–285.

*119.* M. Sandigursky, S. Sandigursky, P. Sonati, M. J. Daly, W. A. Franklin, Multiple uracil-DNA glycosylase activities in *Deinococcus radiodurans*, *DNA Repair*, *3* (2004) 163–169.

*120.* Y. S. Yun, Y. N. Lee, Purification and some properties of superoxide dismutase from *Deinococcus radiophilus*, the UV-resistant bacterium, *Extremophiles*, *8* (2004) 237–242.

*121.* E. Jolivet, E. Corre, S. L´Haridon, P. Forterre, D. Prieur, *Thermococcus marinus* sp. nov. and *Thermococcus radiotolerans* sp. nov., two hyperthermophilic archaea from deep-sea hydrothermal vents that resist ionizing radiation, *Extremophiles, 8* (2004) 219–227.

*122.* M. Mergeay, S. Monchya, T. Vallaeys, V. Auquier, A. Benotman, P. Bertin, S. Taghavi, J. Dunn, D. van der Lelie, R. Wattiez, *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes, *FEMS Microbiol. Rev. 27* (2003) 385–410.

*123.* M. Valls, V. de Lorenzo, Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution, *FEMS Microbiol. Rev. 26* (2002) 327–338.

*124.* C. Schiraldi, F. Marulli, I. Di Lernia, A. Martino, M. De Rosa, A microfiltration bioreactor to achieve high cell density in *Sulfolobus solfataricus* fermentation, *Extremophiles*, *3* (1999) 199–204.

*125.* C. Fuchs, D. Köster, S. Wiebusch, K. Mahr, G. Eisbrenner, H. Märkl, Scale up of the dialysis fermentation for high cell density fermentation of *E. coli*, *J. Biotechnol. 93* (2002) 243–251.

# Biokatalitički potencijal ekstremofila i ekstremozima

**Sažetak**

Ekstremofili su iznimno nekonvencionalni organizmi koji mogu bujno rasti u ekstremnim uvjetima što su formalno nemogući za život. Ekstremni uvjeti mogu biti visoke ili niske temperature, visoki ili niski pH, velika slanost, velika koncentracija metala, vrlo mali udjel hranjivih tvari, vrlo mali aktivitet vode, visoke doze zračenja, visoki tlakovi i nedovoljna količina kisika. Neki ekstremofili žive pod različitim stresnim uvjetima. Ekstremofili su strukturno prilagođeni na molekularnoj razini kako bi preživjeli u tim uvjetima. Biokatalizatori, nazvani ekstremozimima, koje proizvode ti mikroorganizmi su proteini što funkcioniraju pod ekstremnim uvjetima. Zbog njihove specifične stabilnosti, ekstremozimi otvaraju nove mogućnosti za biokatalizu i biotransformaciju. Ekstremozimi obuhvaćaju celulaze, amilaze, ksilanaze, proteaze, pektinaze, keratinaze, lipaze, esteraze, katalaze, peroksidaze i fitaze što imaju veliku mogućnost primjene u raznim biotehnološkim procesima. Danas se komercijalno koristi 1–2 % mikroorganizama, a među njima samo nekoliko ekstremofila. Međutim, ponovno zanimanje za te ekstremofile, kao rezultat novoga načina uzgoja i proizvodnje te uspjeha u kloniranju i ekspresiji njihovih gena u mezofilnim domaćinima, povećat će biokatalitičku primjenu ekstremozima.