

POLAR MICROORGANISMS AND BIOTECHNOLOGY

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INTRODUCTION

Among the various organisms thriving in extreme environments on Earth, psychrophiles (cold-loving organisms) are the most abundant in terms of biomass, diversity, and distribution. If a psychrophile is defined as an organism living permanently at temperatures close to the freezing point of water in thermal equilibrium with the medium (without entering the debate on classification), this definition includes de facto a large range of representatives from all three domains: *Bacteria*, *Archaea*, and *Eukarya* (including yeasts, algae, marine invertebrates, and polar fish). Such biodiversity partly correlates with a less detrimental effect of low temperatures on cellular structures as compared with high temperatures or extreme pH, for instance, but it also reflects the fact that most of Earth's biotopes are cold and have been successfully colonized by diverse organisms (Margesin and Schinner, 1999b; Deming, 2002; Margesin et al., 2002, 2008; D'Amico et al., 2006; Gerday and Glansdorff, 2007). Extreme psychrophiles have been traditionally sampled from Antarctic and Arctic polar regions, as-

suming that low temperatures persisting over a geological time scale have promoted deep and efficient adaptations to freezing conditions. More recently, Arctic permafrost, representing more than 20% of terrestrial soils, has revealed an unsuspected biodiversity in cryopegs, i.e., salty water pockets that have remained liquid for about 100,000 years at -10°C (Gilichinsky et al., 2005). High-altitude mountains, glaciers, and natural caves are additional sources of cold-adapted organisms. However, the largest psychrophilic reservoir is provided by oceans, covering 70% of our planet, which have a constant temperature of 4°C below a depth of 1,000 m, irrespective of latitude. Furthermore, deep-sea sediments, previously considered as abiotic, apparently host a considerable microbial biomass that remains almost uncharacterized as a result of technical difficulties in sampling and culturing (Leigh Mascarelli, 2009).

Such high abundance of psychrophiles evidently offers a huge potential for biomining using culture-based techniques, recombinant protein expression, and metagenomic approaches (Leary, 2008; Lohan and Johnston, 2005). Incidentally, the first cold-adapted enzymes from Antarctic bacteria that have been cloned, sequenced, and expressed in a recombinant form were lipases, subtilisins, and

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α -amylase, i.e., well-known representatives of industrial enzymes. This illustrates that besides the fundamental research on biocatalysis in the cold, the biotechnological potential of psychrophilic enzymes was already put into perspective in the early 1990s. Since then, numerous possible applications based on psychrophiles have been described and patenting in this field is growing (Hoag, 2008). By contrast, the number of known or proven current applications remains modest. It should be stressed that confidentiality accompanying commercial products frequently obscures the possible psychrophilic origin of compounds and, accordingly, the number of current applications is certainly much higher than those summarized below. We present here an overview of the biotechnological uses of psychrophiles and of their biomolecules using selected examples. Previous reviews should be consulted for a complete coverage of this topic (Russell, 1998; Margesin and Schinner, 1999a; Gerday et al., 2000; Allen et al., 2002; Cavicchioli et al., 2002; Marx et al., 2007; Margesin et al., 2008; Margesin and Feller, 2010).

ADVANTAGES OF POLAR MICROORGANISMS IN BIOTECHNOLOGY

While the growth of most microorganisms is stopped or at least severely inhibited in a refrigerator, psychrophiles actively divide at these temperatures. As shown in Fig. 1, some wild-type psychrophilic bacteria display doubling times at 4°C comparable to those of fast-growing *Escherichia coli* laboratory strains grown at 37°C. The latter fail to grow exponentially below 8°C (Strocchi et al., 2006), whereas psychrophilic bacteria maintain doubling times as low as 2 to 3 h at 4°C. This is primarily achieved by a weak effect of low temperature on growth rates of psychrophiles as compared with mesophiles. This efficiency is mainly determined by cold adaptation of the enzymatic machinery (see below). Although contamination of cold-room facilities by psychrophilic or psychrotolerant species is a concern for food spoilage, their growth properties can be advantageously exploited in human-driven operations. Psychrophilic strains, as cell factories, can be grown at tap-water temperatures, avoiding heating of fermentation units,

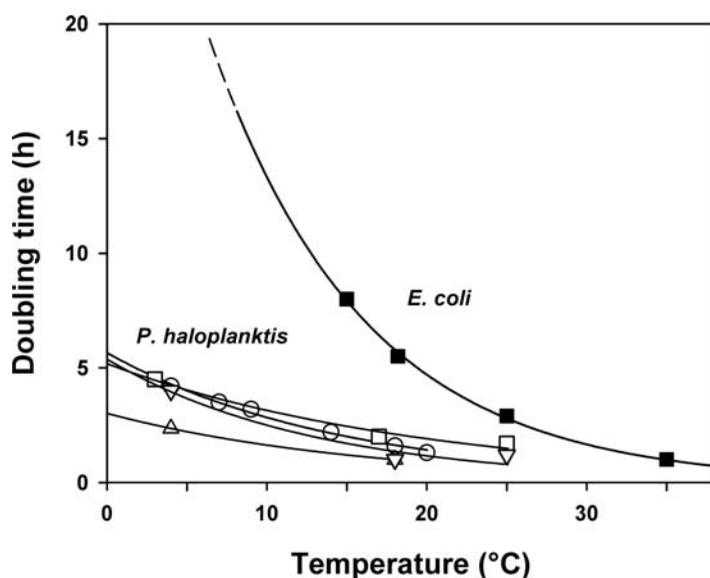


FIGURE 1 Temperature dependence of growth (expressed by the generation time) of *Escherichia coli* and of representative psychrophilic gram-negative bacteria (data from various Antarctic isolates of *Pseudoalteromonas haloplanktis*), displaying high growth rates at low temperatures for the latter.

or at lower temperatures to produce heat-labile compounds and aggregation-prone proteins (Table 1). A promising application also lies in their use in wastewater or soil bioremediation during winter in temperate regions or as bio-additives to relieve pollution (e.g., oil spills) in

cold ecosystems that have an inherently lower propensity to recover.

Microbial adaptation to low temperatures of course requires a vast array of metabolic and structural adjustments at nearly all organization levels of the cell, which have begun

TABLE 1 Selected applications of cold-active biomolecules in biotechnology^a

Application field	Advantage(s)	Involved biomolecule(s)
Gene expression	Recombinant protein expression at low temperature	Chaperonins
Detergents	Washing at low temperature (energy saving, and applicable to synthetic fibers), contact lens cleaning	Protease, lipase, amylase, cellulase, oxygenase
Food industry	Reduced incubation time for lactose hydrolysis in milk and dairy products	β -Galactosidase
	Improved bread quality	Xylanase
	Improved juice clarification, increased juice yield	Pectinase, cellulase
	Efficient and gentle removal of fish skin, meat tenderization	Protease, carbohydrase
	Cold pasteurization, food preservation	Catalase, lysozyme, glucose oxidase, antifreeze proteins
	Improved taste and texture of ice cream	Antifreeze proteins
	Improved taste and aroma of fermentation products (e.g., cheese, dry sausages, alcoholic beverages)	Enzymes involved in fermentation and ripening
Organic synthesis	Synthesis of volatile and heat-sensitive compounds (e.g., flavors and fragrances)	Lipase, esterase, protease, etc.
	Synthesis of acrylamide	Nitrile hydratase
	Organic-phase biocatalysis (increased solvent choice, product yield, and biocatalysis stability)	Enzymes operating under low-water conditions
Molecular biology	Mild heat inactivation of enzymes without interference with subsequent reactions	Various enzymes
	Efficient low-temperature ligation	DNA ligase
	Prevention of carryover contamination in PCR	Uracil-DNA glycosylase
	Rapid 5' end labeling of nucleic acids	Alkaline phosphatase
	Efficient protoplast formation	Cellulase, xylanase, etc.
Pharmaceuticals, cosmetics	Debridement of necrotic tissue, digestion promotion, chemonucleolytic agents	Multienzyme systems, proteases
	Scar treatment, cosmetic creams	Glycoproteins (antifreeze)
Textiles	Improved quality after desizing, biopolishing, and stone-washing of fabrics	Amylase, laccase, cellulase
Biosensors	Selective, sensitive, and rapid online monitoring of low-temperature processes; quality control	Various enzymes
Environment	In situ/on-site bioremediation of organic contaminants	Enzymes involved in biodegradation
	Low-energy wastewater treatment	Enzymes involved in mineralization, nitrification, denitrification, etc.
	Low-energy anaerobic wastewater treatment	Enzymes involved in anaerobic biodegradation
	Low-temperature biogas (methane) production	Enzymes involved in anaerobic biodegradation
	Low-temperature composting	Enzymes involved in litter degradation

^aAdapted from Margesin et al. (2007) with permission of the publisher.

to be understood thanks to the availability of genome sequences and of proteomic approaches (Gerday and Glansdorff, 2007; Margesin et al., 2008). A survey of these data shows that the main upregulated functions for growth at low temperatures are protein synthesis (transcription, translation), RNA and protein folding, membrane homeostasis, antioxidant activities, and regulation of specific metabolic pathways. However, the few common features shared by all these psychrophilic genomes and proteomes have suggested that cold adaptation superimposes on preexisting cellular organization, and accordingly, the adaptive strategies may differ between various microorganisms.

BIOPROSPECTING THE POLAR GENETIC RESOURCES

Two reports of the United Nations University Institute of Advanced Studies (UNU-IAS) have described the bioprospecting activities in the Antarctic (Lohan and Johnston, 2005) and in the Arctic (Leary, 2008). The former report stressed that the absence of clear rules governing the use and ownership of genetic resources from Antarctica, resulting from the peculiar international status of the continent, obviously inhibits commercially oriented research and information exchanges. Such concerns have been frequently debated (Williams, 2004). On the other hand, the latter report devoted to the Arctic provides an extensive survey of companies active in this field and of the patents and products derived from Arctic organisms. This survey clearly demonstrates the potential of psychrophiles in an unsuspected wide range of applications and the intense commercial activity in the field. More recently, UNU-IAS has launched Bioprospector, an online database (<http://www.bioprospector.org/bioprospector/>) surveying patents, commercial products, and companies involved in applied research using genetic resources from both the Antarctic and the Arctic. This excellent initiative, accompanied by relevant publications, is currently the most updated survey of biotechnological applications based on psychrophiles.

The main areas of interest in terms of investigation, patenting, and commercial products are ranked below (Lohan and Johnston, 2005; Leary, 2008), and some relevant examples are given in the next sections (see also Table 1).

- Enzymes: their use in a wide range of industrial processes including food technology, as well as laboratory reagents in molecular biology or medical research.
- Biomolecules: generally as food additives such as dietary supplements for use in aquaculture, livestock, and human diets, with special focus on polyunsaturated fatty acids and on antifreeze proteins.
- Pharmaceutical and medical uses: mainly focused on screening for new antibiotics or anticancer drugs (Biondi et al., 2008), but also on cosmetics and nutraceuticals.
- Bioremediation: applying biostimulation or bioaugmentation to degrade pollutants with cold-adapted microorganisms following accidental spills or to address past waste disposal practices.

POLAR BACTERIA AS CELL FACTORIES

To facilitate biotechnological applications of psychrophiles and of their products, recombinant protein secretion systems efficiently working at low temperature are indispensable. The production level of cold-active (heat-labile) proteins by wild-type strains is usually too low for production on an industrial scale. The first recombinant production of a cold-active enzyme (α -amylase from Antarctic *Pseudoalteromonas haloplanktis*) in an Antarctic host bacterium of the same species was described in 2001 (Tutino et al., 2001). The cold gene-expression system was further developed and optimized for the recombinant extracellular secretion of heterologous proteins in *P. haloplanktis*, with enzymes originating from various Antarctic *P. haloplanktis* strains and a mesophilic yeast (Cusano et al., 2006; Papa et al., 2007). The simultaneous secretion of proteolytic enzymes that degraded the recombinant products could be considerably reduced by inactivating the secretion system with the use of

a gene insertion strategy; the mutant strain still secreted the cold-active enzyme (α -amylase) as efficiently as the wild type and in a stable form (Parrilli et al., 2008).

Another recombinant protein expression system working at low temperature was developed by using an Antarctic *Shewanella* sp. strain (Miyake et al., 2007) and was based on the selection of a suitable promoter and a broad-host-range plasmid. High yields of β -lactamase were produced in the *Shewanella* sp. strain at 4°C; the enzyme yield produced at 4°C was 64% of that obtained at 18°C. The efficiency of the system was demonstrated by the production of foreign proteins (putative peptidases and a glucosidase) from the psychrophile *Desulfotalea psychrophila*.

Site-specific mutants of psychrophiles are a useful tool to study cold adaptation and expression of cold-active enzymes at low temperature (Bakermans et al., 2009). For example, the role of a substrate-binding subunit of a specific transporter in a Siberian psychrophile *Psychrobacter arcticus* strain in the transport of several substrates at low temperatures could be elucidated.

Contrary to the above-mentioned works, which focused on the construction of cold expres-

sion systems for *Proteobacteria*, an expression system was developed for high-G+C gram-positive bacteria that are known to occur frequently in cold environments (Miteva et al., 2008). Seven psychrophilic isolates from Greenland ice cores belonging to various genera of the class *Actinobacteria* (*Arthrobacter*, *Microbacterium*, *Curtobacterium*, and *Rhodoglobus*) were transformed with a shuttle vector that was constructed by using a small cryptic plasmid from a psychrophile *Arthrobacter agilis* strain and conferred antibiotic resistance. In some isolates, plasmid stability was higher at 5 than at 25°C, which points to the efficiency of the expression system within a restricted low-temperature range.

COLD-ACTIVE ENZYMES IN BIOTECHNOLOGY

In contrast to these variable cellular adjustments, most enzymes from psychrophiles are cold active, and this peculiarity provides the basis for the main physiological adaptation to low temperatures. Indeed, cold-active enzymes allow the persistence of metabolic fluxes compatible with sustained growth at freezing temperatures. As shown in Fig. 2, psychrophilic

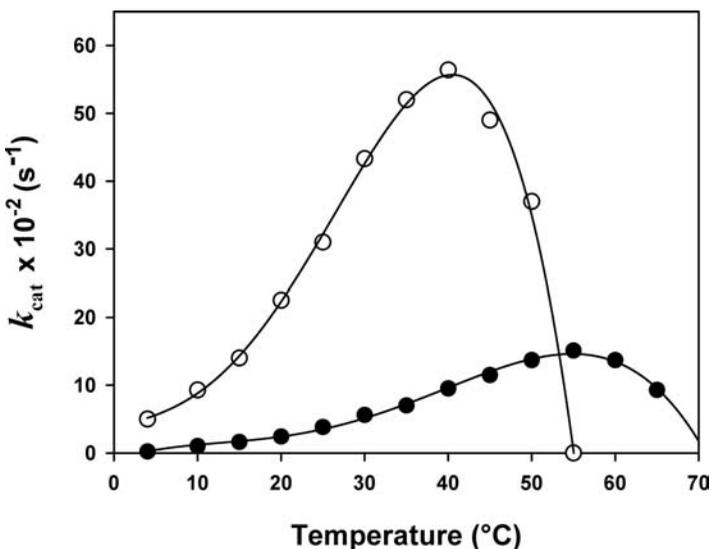


FIGURE 2 Temperature dependence of the activity of psychrophilic (○) and mesophilic (●) enzymes recorded at various temperatures illustrates the main properties of cold-adapted enzymes: cold activity and heat lability (data for cellulases from the Antarctic bacterium *Pseudoalteromonas haloplanktis* and from the mesophile *Erwinia chrysanthemi*). (Adapted from D'Amico et al. [2006] with permission of the publisher.)

enzymes can be up to 10 times more active at low and moderate temperatures as compared with their mesophilic homologues. Furthermore, psychrophilic enzymes are heat labile and are frequently inactivated at temperatures that are not detrimental for their mesophilic counterparts. These specific traits are responsible for the three main advantages of cold-active enzymes in biotechnology: (i) as a result of their high activity, a lower concentration of the enzyme catalyst is required to reach a given activity, therefore reducing the amount of costly enzyme preparation in a process; (ii) as a result of their cold activity, they remain efficient at tap-water or ambient temperature, therefore avoiding heating during a process, either at domestic (e.g., washing machine) or industrial levels; and (iii) as a result of heat lability, they can be efficiently and sometimes selectively inactivated after a process by moderate heat input. Besides these traits specifically linked to temperature adaptation, an additional important aspect has to be mentioned: enzymes from organisms endemic to cold environments can be a valuable source of new catalysts possessing useful enzymological characteristics such as novel substrate specificities or product properties, as exemplified by lipases from the yeast *Candida antarctica* or by the xylanase from the bacterium *P. haloplanktis* (see below).

The conformations and three-dimensional structures of psychrophilic proteins are not markedly different from their mesophilic homologues, and furthermore all amino acid side chains that are essential for the catalytic mechanism are strictly identical. It was found, however, that cold-active enzymes maintain the appropriate flexibility and dynamics of the active site at temperatures at which their mesophilic and thermophilic counterparts have severely restricted molecular motions (D'Amico et al., 2003b; Feller and Gerday, 2003; Tehei et al., 2004). This is achieved by the disappearance of discrete stabilizing interactions either in the whole molecule or at least in structures adjacent to the active site. Among these destabilizing factors, the most relevant are a reduced number of proline residues and of electrostatic

interactions (ion pairs, H-bonds, aromatic interactions), a weakening of the hydrophobic effect, the strategic location of glycine residues, an improved interaction of surface side chains with the solvent, and an improved charge-induced interaction with substrates and cofactors (Smalås et al., 2000; Siddiqui and Cavicchioli, 2006). This adaptive destabilization of psychrophilic enzymes has been demonstrated to be responsible for both cold activity and low thermal stability (D'Amico et al., 2001, 2003a).

INDUSTRIAL ENZYMES FROM POLAR MICROORGANISMS

At the industrial level, the best-known representative of polar microorganisms is certainly the yeast *Candida antarctica*, as its species name unambiguously refers to the sampling origin. This yeast produces two lipases, A and B, the latter being sold, for instance, as Novozym 435 by Novozymes (Bagsvaerd, Denmark). Although the moderate heat stability of this lipase in aqueous solutions can be of concern, this enzyme is stabilized in its immobilized form. As a result of its substrate and stereospecificity, lipase B is involved in a very large number of organosynthesis applications related to food/feed processing, pharmaceuticals, and cosmetics (Babu et al., 2008). In a survey of patents related to Antarctica (Lohan and Johnston, 2005), it was shown that lipases from *C. antarctica* by far dominate the number of process- or product-based patents. This is a significant example of the potential for novel catalysts from genetic resources in cold environments.

The market for enzymes used in detergents represents 30 to 40% of all enzymes produced worldwide. Among these enzymatic cleaning agents, subtilisin (an alkaline serine protease predominantly produced by *Bacillus* species) dominates this market. At the domestic level, however, the current trend is to use detergents at lower washing temperatures because of the associated reductions in energy consumption and costs, as well as to protect the texture and colors of the fabrics. Accordingly, cold-active subtilisins are required for optimal washing

results at tap-water temperatures, and the current advertisements for cold-active detergents indicate that this goal has been reached. The first psychrophilic subtilisins isolated from Antarctic *Bacillus* species have been extensively characterized to comply with this requirement (Davail et al., 1994; Narinx et al., 1997). However, they suffered from a low heat stability that can compromise their storage, and also from a low chemical stability in the detergent components. Therefore, subtilisins currently incorporated in cold-active detergents are engineered enzymes that combine storage stability, alkaline stability, and activity and cold activity. Although psychrophilic subtilisins are not components per se of cold-active detergents, they have largely contributed to the advancement of this economically attractive concept.

The xylanase from the Antarctic bacterium *P. haloplanktis* is a good example of the successful biotechnological transfer from academic research to industry. Xylanases are glycoside hydrolases that degrade the polysaccharide β -1,4-xylan, thus breaking down hemicellulose, one of the major components of plant cell walls. Xylanases are also a key ingredient of industrial dough conditioners used to improve bread quality. It was found that the Antarctic enzyme belonged to a new class of xylanases as both its amino acid sequence and fold were distinct from previously characterized xylanases. The psychrophilic enzyme was therefore subjected to intensive investigations aimed at elucidating the structural origins of its high cold activity and weak stability as well as understanding its enzymological mode of action (Collins et al., 2002, 2003, 2005; Van Petegem et al., 2003; De Vos et al., 2006). Furthermore, baking trials have revealed that the psychrophilic xylanase is very effective in improving dough properties and final bread quality, with, for instance, a positive effect on loaf volume (Collins et al., 2006). This efficiency appears to be related to the high activity of the psychrophilic xylanase at cool temperatures required for dough resting and to its specific mode of xylan hydrolysis. Fol-

lowing careful production optimization of this peculiar xylanase, the product is now sold by Puratos (Groot-Bijgaarden, Belgium). This is apparently the psychrophilic enzyme produced at the highest weight amounts to date.

β -Galactosidase, or lactase, is also a glycoside hydrolase that specifically hydrolyzes the milk sugar lactose into galactose and glucose. It should be stressed that 75% of the world population suffers from lactose intolerance arising from deficient synthesis of intestinal lactase in adults and resulting in digestive disorders due to fermentation of lactose by enteric bacteria. In this context, a cold-active lactase from an Antarctic bacterium has been patented for its capacity to hydrolyze lactose during milk storage at low temperatures (Hoyoux et al., 2001). It is worth mentioning that commercially available lactases require milk heating to become active. This heating step has, however, detrimental effects on milk quality as it alters the aspect, taste, and texture (by Maillard reactions, activation of proteases, coagulation, etc.). Although the psychrophilic lactase is apparently not used for this specific application, it is expected that it will be produced soon in large quantities by Nutrilab (Bekkevoort, Belgium) to hydrolyze lactose (a by-product of the dairy industry) in the processing of the high-value sweetener D-tagatose, a natural monosaccharide with low caloric value and glycemic index.

POLAR PROTEINS IN MOLECULAR BIOLOGY AND COSMETICS

In pioneering work, Kobori et al. (1984) purified and characterized a heat-labile alkaline phosphatase from an Antarctic bacterium isolated in seawater samples from McMurdo Sound. Alkaline phosphatases are mainly used in molecular biology for the dephosphorylation of DNA vectors prior to cloning to prevent recircularization, for the dephosphorylation of 5'-nucleic acid termini before 5' end labeling by polynucleotide kinase, or for removal of dNTPs and pyrophosphate from PCR reactions. However, the phosphatase has to be carefully removed after dephosphorylation

to avoid interference with the subsequent steps. Furthermore, *E. coli* and calf intestinal alkaline phosphatase (which was the preferred enzyme for these applications) are heat stable and require detergent addition for inactivation. It follows that heat-labile alkaline phosphatases are excellent alternatives as they are inactivated by moderate heat treatment, allowing performance of the subsequent steps in the same test tube and minimizing nucleic acid losses. While the scientific report of Kobori et al. (1984) specifically stressed the usefulness of their heat-labile alkaline phosphatase as a new tool in molecular biology, this interesting finding was apparently not turned into a marketed product, possibly because gene cloning and heterologous expression were not well established at that time. Fifteen years later, the group of Bouriotis isolated an alkaline phosphatase from another Antarctic bacterium and cloned its gene in *E. coli* (Rina et al., 2000), solved its crystal structure (Wang et al., 2007), and also showed that its properties can be further improved by directed evolution in terms of high activity and heat lability (Koutsoulis et al., 2008). This heat-labile alkaline phosphatase, sold as Antarctic phosphatase, is now marketed by New England Biolabs (Ipswich, MA).

Cold-active chaperones have also found very useful application in the production of recombinant proteins. High-level expression of heterologous proteins in *E. coli* can result in the production of large amounts of incorrectly folded proteins, generating aggregates of inactive protein generally in the form of inclusion bodies. To circumvent this insolubility problem, low-temperature cultivation of *E. coli* represents a classical strategy and coexpression of chaperones also frequently improves the recovery of soluble proteins. Chaperones are a ubiquitous class of proteins that assist the folding of nascent polypeptides, preventing or even repairing misfolding. In this context, the chaperonins Cpn10 and Cpn60 (homologous to GroES and GroEL in *E. coli*) from the Antarctic bacterium *Oleispira antarctica* were shown to improve the growth of *E. coli* at low

temperatures and to remain optimally active as folding catalysts at these low temperatures (Ferrer et al., 2003). Taking advantage of these properties, the ArcticExpress *E. coli* cells from Agilent Technologies (Santa Clara, CA) have been engineered to coexpress the cold-active chaperonins with the recombinant protein of interest, therefore improving protein processing at low temperatures and increasing the yield of active, soluble recombinant protein.

To conclude this section with beauty, a cosmetic additive is worth mentioning. Antarcticine-NF3 is a glycoprotein with anti-freeze properties produced by the bacterium *Pseudoalteromonas antarctica*, which has been patented by Spanish researchers (Parenta Duena et al., 2006). It was found that Antarcticine is effective for scar treatment and re-epithelialization of wounds. This glycoprotein is now included in some cosmetic regeneration creams (sometimes under the name Antarctilyne). It is also proposed for use in association with edelweiss extract: this is of course reminiscent of the peculiar resistance to harsh conditions of both the Antarctic bacterium and the Alpine flower.

HYDROCARBON BIOREMEDIATION IN POLAR ENVIRONMENTS

The capacity of a broad spectrum of microorganisms to utilize hydrocarbons as the sole source of carbon and energy (biodegradation) was recognized by Zobell in 1946 (Zobell, 1946) and was the basis for the development of biological remediation methods. Bioremediation attempts to accelerate natural biodegradation rates through the optimization of limiting environmental conditions and is an ecologically and economically effective method; nonetheless, it has its limitations. A number of studies have shown the feasibility of bioremediation in polar regions; however, more field studies are needed to confirm that the desired cleanup levels can be reached (Atlas, 2010).

Low-temperature biodegradation of organic contaminants in cold ecosystems is a result of the degradation capacity of the indigenous microbial populations. They transform or mineralize organic pollutants into less

harmful, nonhazardous substances, which are then integrated into natural biogeochemical cycles. Most studies on hydrocarbon bioremediation in polar regions have focused on the treatment of petroleum hydrocarbons, since increased petroleum exploration increases the risk of accidental oil release. The bioremediation potential of microorganisms (Bej et al., 2010) and bioremediation strategies to treat contaminants in polar environments (Filler et al., 2008) have recently been described in detail. Evidence for the biodegradation activity of indigenous microorganisms (Margesin, 2007; Brakstad, 2008; Filler et al., 2008; Bej et al., 2010) in contaminated cold environments is provided by high numbers and activities of hydrocarbon degraders, the prevalence of genotypes with catabolic pathways for the degradation of a wide range of hydrocarbons (Yergeau et al., 2009; Panicker et al., 2010), and high mineralization potentials. The majority of these studies are based on polar bacteria; the use of polar fungi for bioremediation (mycoremediation) requires further research (Bej et al., 2010).

Hydrocarbon degraders in polar regions are confronted with special challenges, which—besides reduced enzymatic reaction rates—include increased viscosity of liquid hydrocarbons, reduced volatility of toxic compounds, low levels of nutrients, limited bioavailability of contaminants, and sometimes extremes of pH and salinity (Margesin, 2004; Aislabie et al., 2006; Filler et al., 2008; Bej et al., 2010). Until recently, frozen soils have been considered to be a practically impermeable barrier to pollutants. Meanwhile, studies have confirmed that hydrocarbons can penetrate even into ice-saturated soils (Barnes and Chuvilin, 2009). Microbial activities have been measured at temperatures close to the freezing point of water and in marine ice at temperatures lower than -10°C , indicating that slow hydrocarbon biodegradation occurs in oil-contaminated ice (Brakstad, 2008).

Several remediation schemes have been implemented successfully at petroleum-contaminated sites in the Arctic during the past decade (Mar-

gesin, 2004; Aislabie and Foght, 2008; Filler et al., 2009; Bej et al., 2010). Successful on-site treatments include biopiles (Thomassin-Lacroix et al., 2002) and landfarming, which is now well developed for cold regions and offers low-cost treatment of petroleum-contaminated soils (Paudyn et al., 2008; Walworth and Ferguson, 2008). Engineered bioremediation implies the use of mechanized systems (e.g., forced aeration, heating and insulation systems) and allows remediation of large volumes of petroleum-contaminated soils to cleanup standards within two to three treatment seasons in Alaska (Filler et al., 2006, 2009) and lengthening of the usual short Arctic bioremediation season (June to September) by 3 months (to May to November).

The most widely used bioremediation procedure in polar soils is biostimulation of the indigenous microorganisms by supplementation of appropriate nutrients (and optimization of other limiting factors, such as oxygen content, pH, and temperature); however, care has to be taken to avoid inhibition of biodegradation due to overfertilization (Walworth et al., 2007). Bioaugmentation by inoculating allochthonous hydrocarbon degraders has been used as a bioremediation option to treat petroleum-contaminated sites in Alaska, Canada, Greenland, and Norway. This strategy generally underperformed or gave no better results than fertilization (Margesin, 2004; Filler et al., 2009; Bej et al., 2010). In addition, such inocula are more expensive than commercially available fertilizers. Bioaugmentation with nonindigenous or genetically modified/engineered microorganisms is banned in Antarctica, Norway, Iceland, and Sweden (Filler et al., 2009). The construction of psychrophiles with specific degradative capabilities was reported 20 years ago (Kolenc et al., 1988) and was based on the transfer of the TOL plasmid from the mesophile *Pseudomonas putida* by conjugation to a psychrophile of the same species; the transconjugant degraded toluene at temperatures as low as 0°C . Recently, the gene coding for a monooxygenase involved in the degradation of aromatic hydrocarbons

from the mesophile *Pseudomonas stutzeri* was recombinantly expressed in the Antarctic *P. haloplanktis* (Siani et al., 2006). However, the performance of such strains has still to be proven. Preconditions of a successful application of bioaugmentation are the expression of the biodegrading activities in the polluted environment and the survival of the inoculated strains at least for the time necessary for bioremediation.

WASTEWATER TREATMENT IN COLD ENVIRONMENTS

Bacteria and fungi able to degrade high amounts of organic compounds within a short time at low temperatures represent a promising source as inocula for accelerated wastewater treatment. For example, an Antarctic *Arthrobacter psychrolactophilus* strain displayed all the features necessary for its use as microbial starter, from the viewpoint of both biosafety and production. At 10°C, the strain induced a complete clarification of a synthetic wastewater turbid medium; it hydrolyzed proteins, starch, and lipids and improved the biodegradability of organic compounds in the wastewater (Gratia et al., 2009). Arctic and Antarctic cyanobacteria have been also positively evaluated for wastewater treatment (Chevalier et al., 2000). Another example is low-temperature degradation of phenol, which is the most common representative of aromatic toxic pollutants in a wide variety of wastewaters. Psychrophilic bacteria (*Rhodococcus* spp.) and yeasts (e.g., *Rhodotorula psychrophenolica*) fully degraded up to 12.5 to 15 mM phenol at 10°C under fed-batch cultivation; with some strains phenol degradation occurred even at temperatures as low as 1°C (Margesin et al., 2005). Immobilization may improve phenol degradation by psychrophilic yeast strains (Krallish et al., 2006). Fixed-biofilm reactors inoculated with bacterial consortia were used for the degradation of chlorophenols, and high removal efficiencies were obtained at temperatures down to 4°C (Zilouei et al., 2006). Interestingly, a temperature decrease resulted in a relative increase of the γ -*Proteobacteria* within the consortia. The

biodegradation of polychlorinated biphenyls in polar environments has been reported; however, further studies are needed to successfully implement the treatment of polychlorinated biphenyl-polluted areas (Lo Giudice et al., 2010). A further compound currently of interest is methyl *tert*-butyl ether (MTBE); its extensive use as both an octane enhancer and as an oxygenate in unleaded gasoline over the past decades has led to widespread pollution of surface water and groundwater. An aerobic mixed bacterial culture capable of utilizing MTBE and growing at from 3 to 30°C could be suitable for the cleanup of MTBE-contaminated aquifers. When inoculated into groundwater samples, it degraded MTBE simultaneously with other volatile organic compounds (Zaitsev et al., 2007).

POLAR PLANTS AND ANIMALS IN BIOTECHNOLOGY

Polar plants and animals have also found diverse applications and are worth citing in the context of the present survey. A special mention should be made of the very large number of products derived from the Arctic shrimp *Pandalus borealis* and the Antarctic krill *Euphausia superba*, mainly by Scandinavian and Japanese companies, in research, medical, and pharmaceutical applications (alkaline phosphatase, hydrolase for prevention of immune rejection reactions, hypotensor peptide, anti-inflammatory agent); in food, feed, and beverage processes; and in cosmetics and nutraceuticals (krill oils rich in omega-3 fatty acids). For instance, two shrimp enzymes are marketed for molecular biology applications that take advantage of their heat-labile properties. The heat-labile alkaline phosphatase from the Arctic shrimp *P. borealis* is available from Biotec Pharmacon (Tromsø, Norway) and GE Healthcare Life Sciences (Little Chalfont, United Kingdom) for applications similar to that of the above-mentioned Antarctic phosphatase. Shrimp nuclease selectively degrades double-stranded DNA; it is used for the removal of carryover contaminants in PCR mixtures, and is heat inactivated prior to addition of the template.

This enzyme is produced in recombinant form in *Pichia pastoris* and is available from Biotec Pharmacon, USB Corporation (Santa Clara, CA), and Thermo Scientific (Waltham, MA).

Heat-labile uracil-DNA *N*-glycosylase from Atlantic cod (*Gadus morhua*), which presents typical cold-adaptation features (Leiros et al., 2003), is also used to remove DNA contaminants in sequential PCR reactions. When PCR is performed with dUTP instead of dTTP, PCR products become distinguishable from target DNA, and can be selectively degraded by uracil-DNA *N*-glycosylase. Following degradation of contaminants, the enzyme is completely and irreversibly inactivated after heat treatment. Heat-labile uracil-DNA *N*-glycosylase, produced in recombinant form in *E. coli*, is available from Biotec Pharmacon.

Among polar plants, extracts of the Antarctic alga *Durvillaea antarctica* are included in cosmetics to improve skin vitality, such as in the Extra Firming Day Cream, a top seller of Clarins (Neuilly-sur-Seine, France).

A last example will give a sweet taste for psychrophilic proteins. Most antifreeze proteins are small glycoproteins, first discovered in polar fish (Fletcher et al., 2001), that allow the animals to thrive at subzero temperatures without freezing. As a result of their peculiar surface properties, antifreeze proteins bind to ice crystal seeds and inhibit growth of ice in body fluids that would otherwise be fatal. The precise mechanism of ice adsorption inhibition by antifreeze proteins remains poorly understood because of the complex water-ice interface and the structural diversity of these proteins. Besides this interesting finding, the gene of an antifreeze protein from the ocean pout, an eel-like fish found in northern and Arctic oceans, has been cloned and expressed in the baker's yeast *Saccharomyces cerevisiae*. This recombinant protein is now included in several edible ice cream brands from Unilever (Rotterdam, The Netherlands and London, United Kingdom) under the name of ice-structuring protein. It controls ice recrystallization following thawing-freezing cycles that otherwise drastically reduces taste and texture quality.

Furthermore, the antifreeze protein enables the production of healthier ice creams that are lower in fat and added sugar and with fewer additives. Approval for the use of this technology has been granted by regulatory administrations in many countries.

CONCLUSIONS

Among the extremophiles thriving at extreme biological temperatures, thermophiles have been generally considered as the most promising source of biotechnological innovations. However, recent developments based on cold-adapted organisms and their biomolecules, such as those mentioned here, have clearly demonstrated the huge potential of psychrophiles. This potential appears to be even larger than for thermophiles, considering both the broader psychrophilic biodiversity that encompasses microorganisms, plants, and animals (Margesin et al., 2007) and the broader fields of application. Last but not least, most biotechnological applications of psychrophiles are environmentally friendly and contribute to energy savings, both aspects being of increasing significance.

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