Psychrophiles: Their habitat and applications

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ABSTRACT

Psychrophile bacteria are the extremophilic microorganisms that are able to grow and multiply at low temperatures and have successfully colonized the numerous low temperatures environments on earth. Cold soils play important biogeochemical role in primary biomass production and nutrient cycling, yet little is known about the identity and habitat of microbes active under such conditions. Many microbes are known to be resistant to hostile environmental conditions, and are capable of surviving in harsh environments. Psychrophilic bacteria produce cold-active enzymes and proteins, that have useful applications in molecular biology, medical research, industrial food or feed technology, detergents and cosmetics due to high catalytic activity and heat-lability. Furthermore, they are used for bioremediation of polluted cold soils and waste waters.

Keywords: Psychrophile, extremophile, microbes

INTRODUCTION

Earth primarily being a cold, marine planet with 90% of the ocean's waters at 5°C or lower. Furthermore, permafrost (frozen soils), glaciers and ice sheets, polar sea ice, and snow cover make up 20% of the Earth's surface environments (Deming and Eicken, 2007) thus making psychrophiles the most abundant in terms of diversity, biomass and distribution. Psychrophilic microorganisms have successfully colonized all permanently cold environments from deep sea to mountains. High-altitude cold habitats of the Himalayas are little explored with respect to bacterial diversity. Psychrotolerent microbes are extremely important since they survive and retain their functionality in cold temperature conditions, while growing optimally at warm temperatures. Psychrophilic microorganisms provide an immense natural resource of cold-adapted enzymes that function effectively in the cold, and have been targeted for their biotechnological potential. By providing energy savings to the processes for which these enzymes are used, cold-adapted enzymes can be economically more beneficial and productive than mesophilic or thermophilic homologues at low temperature. In temperate countries where temperature remains very low during winter psychrophilic microorganisms can also be used for the bioremediation of polluted soils and waste waters, when low temperatures impair the degradative capacity of the endogenous microflora.

HABITAT

Cryosphere is not only the integral part of the global climate system but also one of the major habitable ecosystems of Earth's biosphere. As per findings of Priscu and Christner (2004) the total number of bacterial cells in the Antarctic and Greenland ice sheet are to be $9.61x10^{25}$. Glacier ice and snow are interrelated ecosystems because glacier ice is formed from snow as a result of gradual compression and burial for hundrends of thousands of years. Glacier ice is a unique ecosystem preserving microbial life and past climate changes chronologically for hundreds of thousands of years (Margesin *et al*., 2008). Another specific microbial habitat existing on the surface of glacier ice are the cryoconite holes ("cryo" meaning ice and "conite" meaning dust), named by the Swedish explorer A.E. Nordenskjold during his Greenland expedition 1870. Most cryoconite holes are open systems to the surrounding environment. Cryoconite holes have been suggested to play an important role in glacier ecosystems because they contain abundant populations of active living organisms (Wharton *et al*.,1985). It was first proposed by Price (2000) that thin liquid veins exist between ice crystals in ancient polar ice that can provide water, energy and carbon to microbial cells.

Psychrophiles (psychrotolerant or psychrotroph) are the microorganisms that prefer permanently cold environments, but can also tolerate a wide range of temperatures reaching up into the mesophilic range (Caviociolli, 2006). Soil samples taken from different Himalayan habitats showed that majority of cultured bacterial strains belonged to the *Proteobacteria* followed by *Actinobacteria* and *Bacteroidetes*. Gradual decline in the number of genera was observed with increasing altitude. The isolates exhibited close phylogenetic affinities to bacteria from other cold habitats (Gangwar *et al*., 2009). Psychrophilic microorganisms have been studied by culture-dependent and culture-independent methods in permafrost as well. Since the microbial long-term survival in permafrost has been questioned; however, there is evidence that bacteria are able to survive in permafrost that is 500,000-year-old (Gilichinsky *et al*., 2008; Steven *et al*., 2007, 2009; Johnson *et al*., 2007). Soils of alpine regions undergo dramatic temporal changes in their microclimatic properties, suggesting that the bacteria encounter uncommon shifting in selection gradients (Meyer *et al*., 2004). The abundance of total and culturable bacteria deposited into the East Rongbuk glacier, Mt. Everest were investigated, and the bacterial content was examined through culture and culture-independent approaches. Total counts of bacteria in the ice core ranged from $0.02x10^3$ to $6.4x10^3$ cells ml^{-1} (Zhang *et al.*, 2009). Psychrophilic microorganisms are true extremophiles because they are sometimes adapted not only to low temperatures, but also to further environmental constraints. For example, in ocean depths they face extremely high pressure in addition to cold temperature, and are therefore called piezo-psychrophiles (or baro-psychrophiles). The microbial communities comprising of bacteria, algae, fungi and protozoa that are found in sea ice and are exposed to high salt concentrations, are therefore called as halo-psychrophiles (Feller *et al.*, 2003). Some commonly found bacterial phylum in glacial soil are given in table 1.1.

Table 1.1 Some commonly found bacteria in glacier soil

S.No.	Phylum	Source	References
1.	Actinobacteria	China, India, Switzerland	Xiang *et al*. (2005); Gangwar *et al*. (2009); Zumsteg *et al*. (2013)
2.	Proteobacteria	China, India, Switzerland	Xiang *et al*. (2005); Gangwar *et al*. (2009); Zumsteg *et al*. (2013)
3.	Cyanobacteria	Switzerland	Fery *et al*. (2013); Zumsteg *et al*. (2013)
4.	Firmicutes	China, India, Switzerland	Xiang *et al*. (2005); Gangwar *et al*. (2009); Zumsteg *et al*. (2013)
5.	Acidobacteria	Switzerland	Zumsteg *et al*. (2013)
6.	Bacteroidetes	India	Gangwar *et al*. (2009)

COLD-ADAPTED ENZYMES AND THEIR APPLICATIONS

Psychrophiles possess a rare ability to synthesize enzymes that are capable of catalyzing reactions at temperatures that are close to the freezing point of water is no small feat. Low temperatures tend to increase the compactness of proteins and also lead to exponential decreases in chemical reaction rates, thus interfering with the conformational movements necessary for catalysis (Rasmussen *et al.*, 1992). As compared to enzymes derived from mesophilic or thermophilic organisms cold-adapted enzymes have generally been observed to possess high specific activity or catalytic efficiency at low and moderate temperatures. In some cases, cold-adapted enzymes have been observed to catalyze reactions at rates up to one order of magnitude higher than those observed for mesophilic counterparts at low and even moderate temperatures (Fig. 1.1; D'Amico *et al.*, 2006).

The increasing scientific interest in cold-adapted enzymes has increased substantially in recent years, there are number of examples of isolation and characterization of cold-active enzymes from psychrophilic and psychrotolerant organisms. They are beneficial because they can catalyze numerous reactions at low and moderate temperatures (< 40°C) more efficiently and with fewer undesired chemical reactions that may occur at high temperatures, thereby decreasing the overall energy expenditures and processing costs associated with heating steps (Cavicchioli *et al.*, 2002). The biotechnological application of cold-adapted enzymes comes from their high k_{cat} at low to moderate temperatures, high thermolability at elevated temperatures and their ability to function in organic solvents

(Gerday *et al*., 2000; Siddiqui and Cavicchioli, 2006; Marx *et al*., 2007; Margesin and Feller, 2010). The high activity of cold-active enzymes from psychrophilic microorganisms at low and moderate temperatures offers possible economic benefits (Allen *et al*., 2001; Margesin *et al*., 2002; Russel, 1997; Gerday *et al*., 2000; Cavicchioli *et al*., 2002). Biomolecules like unsaturated fatty acids and antifreeze proteins are used generally as food additives such as dietary supplements in human diets, aquaculture and livestock. Psychrophilic microorganisms have successfully been used for bioremediation of organic pollutants in arctic region.

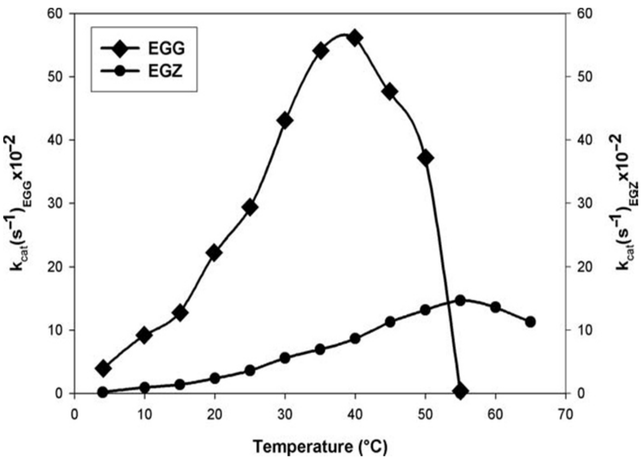

Fig. 1.1. Thermodependence of activity for the cold-adapted cellulase produced by *Pseudoalteromonas haloplanktis* (*EGG*) and its mesophilic homolog produced by *Erwinia chrysanthemi* (*EGZ*); after D'Amico *et al.* (2006).

Enzymes for detergents

Psychrophilic microorganisms produce enzymes (subtilisin, proteases, lipase and glycosidases) that find application in detergents. A major benefit of developing detergents from cold-active enzymes is that it will efficiently hydrolyze soils and stains at low temperatures that would reduce energy consumption resulting in decreased associated costs and environmental impacts. Additionally, garment alterations that take place during warm and hot-water wash cycles, such as fabric degradation, shrinkage and dye bleeding, will be reduced. Cellulases can also be used to clean fabrics indirectly by hydrolyzing glycosidic bonds, thus increasing softness and color brightness in cotton fabrics. Psychrophilic microorganisms that produce cold-active amylases are structurally modified by an increasing flexibility of the polypeptide chain enabling an easier accommodation of substrates at low temperature. Cold-active amylases find their application in detergents at work in cold water (Joseph *et al*., 2008). It is proposed that cold-active lipases from psychrophilic microorganisms can be used in place of mesophilic lipases in detergent industry for the removal of fatty residues in laundry, dishwashers as well as for cleaning of clogged drains in cold waters where later can become inactive (Joseph *et al*., 2008). The use of cold-adapted enzymes may therefore be more economical than mesophilic homologs, as smaller amounts are required to achieve the same effect. Such characteristics of cold-adapted enzymes hold promise for decreasing not only energy requirements of washing but also the costs for manufacturing and use of liquid or granular detergents, stain removers, household cleaners, and industrial cleansing applications (Margesin *et al*., 2008).

Food, pharmaceutical industries and leather industry

Cold-active enzymes find applications in starch and food processing industries due to their high catalytic activity at temperatures that minimize spoilage and alterations in taste and nutritional values (Kuddus *et al*., 2012). In pharmaceutical industry cold active α-amylases can be used as a digestive aid and in food industry for the reduction of haze formation in juices. In brewing industry cold-active α-amylases could be used to speed the mashing phase at low temperatures. In the food industry, approximately two-thirds of the world's population faces problem of lactose intolerance. The lactose from milk can be removed by use of a β-galactosidase from psychrophilic microorganisms. In baking industry psychrophilic glycosidases can be used instead of mesophilic glycosidases to avoid the retention of residual activity after cooking that alters the structure of the final product during storage. Another example, is the use of cold-active pectinases in reducing the viscosity and to clarify fruit juices at low temperatures. In complex mixtures heat-lability of these enzymes also ensures their fast, selective and efficient inactivation. Cold-adapted proteases can also be used for tenderization and taste improvement of refrigerated meat products (He *et al*., 2004), and removal of undesirable tissues from seafood, such as the de-scaling and removal of fish skin and extraction of carotenoproteins from shellfish (Bjarnasen and Benediktsson, 2001; Shahidi and Kamil, 2001). Heat-labile lipase from *Candida antarctica* has found a broad range of applications, in modification of desymetrization of complex drug intermediates polysaccharides and resolution of alcohols and amines (Suen *et al.*, 2004). Furthermore, the sterospecificity of cold-adapted enzymes may be useful for synthesizing chiral drugs which are twice as potent as a racemic mixture (Jeon *et al*., 2009a).

Cold-active proteases are used in industrial peeling of leather, which requires heating of water to 37°C for the process to be performed by mesophilic enzymes instead it can be done at ordinary temperature of water without heating by using cold-active enzymes (Rao *et al*., 1998).

Bioremediation

Oil and fuel spills are among the most extensive and environmentally damaging pollution problems in cold regions and are recognized as potential threats to human and ecosystem health. It is generally thought that spills are more damaging in cold regions, and that ecosystem recovery is slower than in warmer climates (AMAP 1998; Det Norske Veritas, 2003). Psychrophilic microorganisms have been used as a bioremediation option to treat petroleum contaminated sites in Alaska, Canada, Greenland, and Norway. Bacteria and fungi able to degrade high amounts of organic compounds within a short time at low temperatures represent a promising source of technology for accelerated wastewater treatment. For example, a cold-adapted *Arthrobacter psychrolactophilus* strain displayed all the features necessary for its use as microbial starter, both from the viewpoint of biosafety and production (Siani *et al.*, 2006).

Biofuels

With the growing human population there is a continuous growing demand for alternative sources of energy. Psychrophiles have effectively been used for the purpose of improving efficiency in biogas digesters for generation of cooking and heating gas for Alaskan households. Methanogens responsible for biogas production are limited in application to warm environments.

Psychrophil ic methanogens were discovered to improve the biogas production of biogas digesters in cold climates (ACEP, 2004). Cold-adapted glycosyl hydrolases such as cellulases, xylanases and glucosidases may enable cost-effective lignocellulose biomass conversion, thus facilitating the development of an economically-viable and renewable source of fuel to meet the world's increasing energy demands.

Table 1.2 Application of cold active enzymes.

Source	Enzyme / micro-ogranisms	Use	Reference
Psychrophiles	Protease	Contact lens cleaning solution, meat tenderizing, Industrial peeling of leather	Cavicchioli *et al*. (2006); Wang *et al*. (2010a)
	Protease, lipase, cellulases, amylases	Detergent	Joseph *et al.* (2008); Collins *et al.* (2005); Wang *et al.* (2010a)
	Alkaline phosphatase	Molecular biology	Dahiya *et al*. (2006)
	Lipases and proteases	Cheese manufacture	Cavicchioli *et al*. (2006)
	β - Galactosidase	Lactose hydrolysis in milk products	Cavicchioli *et al*. (2006); Joseph *et al*. (2008)
	Microorganism (*Arthobacter psychrolactophius*)	Degradation of organic pollutants	Gratia *et al*. (2009)
	Microorganisms (methanogens)	Biogas production	ACEP(2004)

CONCLUSION

The state of Jammu and Kashmir has a great potential for cold-active enzyme extraction from psychrophilic microorganisms since the state experiences cold climate and has number of glaciers. The discovery of new psychrophilic and genetic engineering of the newly isolated as well as of the currently available extreme microbes will offer novel opportunities for biocatalysis and biotransformations in biotechnology. Cold-adapted enzymes can be exploited to replace the enzymes derived from mesophilic organisms as the former have more economic benefits by decreasing the industrial energy expenditures and costs then the later.

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