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An overview of the biotechnological applications of bacterial cold active enzymes

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Abstract

Bacteria are adapted to survive at extremely low temperatures due to their exceptionally versatile metabolic capabilities. Besides other metabolites, a number of cold active enzymes are also produced by these tiny organisms to make their survival possible at subzero temperatures. Many of these enzymes such as lipase, protease, amylase, cellulase, xylanase etc have wide range of industrial applications. Cold active enzymes have high catalytic activity at lower temperatures as they are able to undergo conformational changes for catalysis with lower energy demand which enhances their applicability for industrial purposes. This review describes the biotechnological and industrial prospects of bacterial cold active enzymes.

Keywords*: bacteria, cold active, enzymes, industrial applications*

Introduction

A large part of the earth's surface is covered by low temperature environments such as north and south poles, deep oceans, snow-covered mountains and glaciers (D'Amico *et al.,* 2006). Given the fact that nearly 71% of the earth's surface being covered by oceans with the temperature of 90% of its total volume measuring below 5^0C, the deep sea covers the major fraction of cold environment on earth (Margesin *et al.,* 2011). This is followed by snow (35% of land surface), permafrost (24% of land surface), sea ice (13% of the earth's surface) and glaciers (10% of land surface). Other cold environments include cold water lakes, soils and deserts. Diverse form of microorganisms like bacteria, archaea, yeast, filamentous fungi and algae are capable of surviving under the extreme conditions prevalent at these cold regions round the year. To describe such cold adapted microorganisms, the term 'psychrophile' was first used in 1902 (Helmke *et al.*, 2004). Presently, cold adapted microorganisms are broadly categorized into two groups "psychrophiles" and "psychrotolerants". After initial debate and arguments among scientists over the description of these terms, the problem was resolved by way of discriminating them on the basis of cardinal growth temperature that includes upper and lower temperature limits with optimal growth temperature (Morita 1975). Therefore, psychrophiles are defined as those organisms which are specifically adapted to low-temperature growth, not only at 0^0C but below it and have optimal and upper limits of growth temperatures below 15 and 20^0C, respectively. On the other hand, psychrotolerants or psychrotrophs are able to survive at 0^0C but grow optimally at 20-25^0C and may have upper growth limit of temperatures as high as 40^0C. In addition to low temperature stresses, some cold adapted microorganisms are well adapted to other environmental constraints like high pressure and salt concentrations, radiation etc. Baropsychrophiles (piezo-psychrophiles), for examples are the group of cold adapted organisms that can withstand high pressure in ocean bottoms (Yayanos, 1995). Similarly, bacterial community inhabiting sea ice can tolerate high salt concentration as well and termed as 'halo-psychrophiles' (Staley *et al.,* 1999). Bacterial community at glaciers is constantly exposed to ultraviolet radiation (Carpenter *et al.,* 2000). Troglo-psychrophiles are capable of inhabiting alpine cave and cracks with low water and nutrients availability in the absence of light (Friedmann

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1982). So it can be said that psychrophiles are the true extremophiles.

Cold adaptations in psychrophilic bacteria

Psychrophilic bacteria are adapted to lower temperature by incorporating a variety of structural and biochemical modifications in cellular machinery. The fatty acid composition of cell membrane affects an organism's ability to withstand cold shock. An abundance of unsaturated, polyunsaturated and methyl branched fatty acids has been noticed in the cell membrane of psychrophilic/psychrotolerant microorganisms. Along with it, cis-unsaturated double bonds and anteiso-branched fatty acids are also present (D'Amico *et al.,* 2006). The presence of these fatty acids helps to increase the membrane fluidity by introducing steric constraints. In mesophilic/ thermophilic organisms, low temperature inhibits various primary cell processes like transcription and translation by reducing the activity of enzymes involved in such reactions but psychrophilic bacteria are endowed with those that are optimally active under cold conditions (Lim *et al.,* 2000). Other proteins like cold-shock proteins (Csps) and heat shock proteins (Hsps) play an important role in bacterial survivability under extreme conditions. Moreover, the ability of psychrophilic bacteria to produce anti-freezing proteins and exopoly saccharides (EPSs) enable them to cope up with extreme cold conditions (Jia *et al.,* 2002, Krembs *et al.,* 2002, Nichols *et al.,* 2005).

Biotechnological applications

Past few decades have witnessed the realization of the vast biotechnological potential of psychrophilic bacteria offering numerous economic and ecological advantages along with their respective enzymes (Kasana *et al.,* 2011). At present there is a global race to reduce energy consumption and in this regard enzymes form psychrophilic bacteria have created new rays of hope for various industries (Margesin, 2010). Cold active enzymes have high catalytic efficiency at lower temperatures as they are able to undergo conformational changes for catalysis with lower energy demand. This significantly reduces the heating requirement in industries (Arpigny, *et al.,* 1997). Moreover cold active enzymes provide increased reaction yields, accommodating high levels of stereo-specificity, high K_{Cat} at low to moderate temperature and thus minimizing the chance of unwanted chemical reactions that might occur at high temperatures (Gerday *et al.,* 2000, Cavicchioli *et al.,* 2002, Margesin *et al.,* 2010). A large number of bacterial cold active enzymes have been isolated and characterized by various scientists and some of them are mentioned in Table 1. The applications of cold active enzymes in various industries are described below.

a) Detergent industry

The inclusion of cold active enzymes in detergent formulations for laundry and dishwasher offers the special advantage of the facility to carry out the entire task at lower temperatures (Aehle, 2007). Increasing demands and commercial requirements have stimulated the use of cold active enzymes at industrial scales too. For example, the food processing plants rely on the detergent with cold adapted enzymes for cleaning of equipments as they reduce the need of cool and hot temperature cleaning cycles, consequently reducing the cost and saving energy and time. They are also used in spray cleansers for large surfaces like buildings, furniture, carpets etc. (Hasan *et al.,* 2010). Compounds such as surfactants and a variety of enzymes, including proteases, amylases and lipases, facilitate the stain removal from fabrics and hard surfaces like dishes. The low wash temperature reduces the energy consumption which is significantly recognized by detergent manufacturer.

It was reported that 30% reduction in electricity use was found with reduction of wash temperature from 40^0C to 30^0C (Nielsen 2005). Besides the energy saving aspect, cold active enzymes also reduce the risk of alterations such as quality degradation of fabric, shrinkage and dye bleeding that may take place during cold and hot water wash cycles. Cellulase is one of the most interesting enzymes which can be used to clean fibers as it hydrolyzes glycosidic bonds and consequently increase color brightness and softness in cotton fabrics. Other enzymes like protease, lipase and amylase are extensively used for washing at low temperature (Aehle, 2007). Cold active lipase and glucose hydrolase is widely used in cleaning solutions to remove mould associated with the old historical monuments and buildings (Valentini *et al.,* 2010). Currently many companies have developed detergent formulations with enzymes that withstand at lower to medium temperature.

Table 1. Cold adapted enzymes and their applications

Enzyme	Source Microorganism	Applications	Reference
α- Amylase	*Bacillus,*	Detergent, pulp bleach, dough fermentation	Groudieva *et al.*, 2004
Aliphatic Aldehyde dehydrogenase	*Cytophaga sp. KUC-1*	Biodergadation	Yamanaka *et al.*, 2002
Alkalin phosphatase	*Vibrio sp.* G15-21	Molecular biology	Jonas *et al.*, 2000
Alcohol dehydrogenase	*Moraxella sp.* TAE 123	Asymmetric chemical synthesis	Tsigos *et al.*, 1998
β-Galacctosidase	*Pedobacter cryoconitis sp. nov*	Dairy Industries (Lactose hydrolysis in milk product)	Margesin *et al.*, 2003
β-Mannanase	*Glaciozyma antarctica PI12, Flavobacterium sp.*	Pulp and paper, pharmaceutical, food, feed, oil and textile industries	Parvizpour *et al.*, 2014
Chitinases	*Arthrobacter sp* strain TAD20	Chitin degradation, food and health products	Lonhienne *et al.*, 2001
Cellulase	*Pseudoalteromonas haloplanktis*	Animal feed textiles, detergent	Violot *et al.*, 2003
DNA ligase	*Pseudoalteromonas haloplanktis*	Molecular biology	Georlette *et al.*, 2000
Esterase	*Psychrobacter cryohalolentis* K5T	Detergent additives, food processing, environmental bioremediation,	Vlasova *et al.*, 2012
Lipase	*Psychrobacter okhotskensis*	Detergent additives, food processing, environmental bioremediation	Yumoto *et al.*, 2003
Lactate dehydrogenase	*Vibrio marinus* MP-1	Clinical biochemistry, pharamceuticals and nanotoxicology	Mitchell *et al.*, 1985
Protease	*Colwellia sp.* NJ341	Detergent, food, molecular biology	Wang *et al.*, 2008
Malate synthase	*Colwellia maris*	Biotransformation	Watanabe *et al.*, 2001
Pectate lyase	*Pseudoalteromonas haloplanktis* ANT/505	Textile processing, wastewater treatment, fruit and vegetable processing	Truong *et al.*, 2001
Pullulanase	*Shewanella arctica* 40-3	Starch processing industries, Baking industries	Qoura *et al.*, 2014
Xylanase	*Flavobacterium frigidarium* sp. nov.	Dough fermentation, wine and juice industries	Humphry *et al.*, 2001
RNA polymerase	*Pseudomonas syringae*	Molecular Biology	Uma *et al.*, 1999

Some examples of washing products involving cold active enzymes are summarized in Table 2.In recent times scientific communities are keenly interested to explore the psychrophilic bacteria with ability to produce potent cold active enzymes. *Serratia rubidaea* and *Stenotrophomonas maltophilia* have been reported as potent protease producing psychrophilic bacteria (Cavicchioli *et al.*, 2011). Metagenomic studies also provide promising results as intensive studies have been carried out to screen potent cold adapted genes. (Sharma *et al.*, 2010).

Table 2. Commercial detergents with cold active enzymes

S.N.	Products	Company	Enzyme incorporated	Wash Temperature
1	Kannase®	Novozymes	Protease	10–20°C;
2	Celluzyme®	Novozymes	Cellulase	Below 15°C
3	Savinase®	Novozymes	Alkaline protease	(45–55°C)
4	Purafect®	Genencor	Protease	NA
5	Properase®	Genencor	Protease	NA
6	Puradax®	Genencor	Cellulase	NA

NA = Not available

b) Food industry

Food processing at lower temperature minimizes spoilage and alterations in taste and nutritional values of food. The product manufacturing process is made cost effective by cold active enzymes as after attaining the desired flavor and texture of product, the process can be easily stopped owing to low structural stability of cold active enzymes. Cold active milk coagulating enzymes have the advantage of controlled casein coagulation for maintaining the quality of whey which can be used in other processes.Their use is also helpful in minimizing the cost of cheese production as the cold active proteases and lipases replace the calf rennet and accelerate the maturation of slow-ripening cheeses that require specific low temperature and low-moisture conditions. Rennet from cold loving microorganisms is commercially available in market with brand names as Marzyme, Rennilase 50TL and Modilase (Ramana *et al.*, 2000). Cold active proteases are also used to enhance the flavor and tenderization of refrigerated meat, removal of undesired tissue and for de-scaling of fishes (Shahidi *et al.*, 2001, He *et al.*, 2004). In baking industries cold adapted enzymes are frequently used for various purposes. Simultaneous action of enzymes like amylase, protease, lipase, xylanase, and glucose oxidase help improve elasticity and machinability of dough. Lactose hydrolyzing enzyme β-galactosidase is also important in food industry as its treatment results in increased solubility, digestibility and sweetness of milk. Use of cold active β-galactosidase for lactose hydrolysis can increase yield up to 70-80% as its mesophilic counterpart is generally active at 30-40^{0}C and at this temperature milk is easily spoiled by other microorganisms. Ability of β-galactosidase to operate at acidic pH is helpful in decreasing the pollution impact of whey, which if left untreated may lead to growth of harmful microorganisms. This byproduct of milk industry can be broken down into glucose and galactose by β-galactosidase for subsequent use as sweetener agents in various food products and substrates for alcohol production (Gerday *et al.*, 2005). Another important cold active enzyme is pectinase which is widely used for fruit juices and cheese processing at lower temperature (Nakagawa *et al.*, 2004).

c) Pharmaceutical industries

Pharmaceutical industries have huge applications of cold adapted enzymes due to their ability to catalyze reactions at lower or ambient temperatures. The ability of cold adapted enzymes to withstand aqueous, nonaqueous and organic solvents without limiting the conformation mobility makes them more suitable for pharmaceutical industries. (Owusu-Apenten 1999, Schoemaker *et al.*, 2003). Psychrophilic bacteria are the natural source of various cold active enzymes and other pharmaceutically important compounds. Various psychrophilic bacterial strains belonging to the

genera of *Streptomyces, Alteromonas, Bacillus, Micrococcus, Aeromonas, Flavobacterium, Moraxella, Pseudomonas* and *Vibro* have been reported with this potential (Ramana *et al.,* 2000). Cold active lipase is extensively used for trans-esterification and hydrolysis reactions in pharmaceutical industries (Joseph *et al.,* 2007). Production of specialty lipids and digestive acids become more feasible with the utilization of cold active lipase (Vulfson 1994). Modification of monoglycerides which is used as emulsifier in pharmaceutical industries is carried out using cold active lipase (Sharma *et al.,* 2001). For biotransformation of different bioactive compounds such as flavoring agents, lipase is used as stereo-specific catalyst in pharmaceutical industries (Adrio *et al.,* 2014). Marine psychrophilic bacteria also provide a huge range of biologically active compounds. Previous studies revealed that marine bacteria are rich source of anti-tumor, anti-viral compounds and polyunsaturated fatty acids (PUFA) (Joseph *et al.,* 2007, Yada *et al.,* 2008).

d) Bioremediation

Various studies regarding biodegradation at ambient temperature have been undertaken in the past, but research in cold regions is still in its infancy due to lack of information regarding involvement of specific microbes, genes, biochemical pathways and enzymes involved. Cold environment really increases the challenge of bio-degradation as lower temperature directly influences the rate of biodegradation. However, various cold adapted microorganisms have been reported having roles in biodegradation of pollutants (Margesin *et al.,* 1998, Whyte *et al.,* 1999, Mohn *et al.,* 2001). Bacterial oxidoreductase is one of the most fascinating enzymes used for detoxification of toxic organic compounds through oxidative coupling (Gianfreda, *et al.,* 1999). The enzyme helps cleave the chemical bonds and support the electron transfer from reduced organic substrate to another chemical compounds as an electron acceptor. This process of oxidation and reduction neutralizes toxic effect of the pollutant (Karigar *et al.,* 2011). Oxidoreductases can detoxify the phenolic and anilinic compounds through polymerization and copolymerization with other substrate or by binding through humic substances (Park *et al.,* 2006). *Flavobacterium sp.* HK1D9 and *Arthrobacteria sp.* MPS8D3 isolated from Antarctica have been reported as efficient oxidoreductase producers (Araujo *et al.,* 2011). Cold active peroxidase also has distinctive properties that can be exploited for bioremediation. Peroxidase is actively involved in oxidation of lignin and other phenolic compounds but it needs peroxides such as hydrogen peroxides (H_2O_2) for activation. There are different types of peroxidases like horseradish peroxidase which is frequently used for enzymatic waste water treatment (Spadiut, 2013). A variety of aromatic compounds such as phenols, benzidines, biphenols can be oxidized using horseradish peroxidase after H_2O_2 activation (Karam *et al.,* 1997). The cost effective eco-friendly method of waste water treatment is use of cold active pectinases, which helps to remove pectic substances from waste water and make the decomposition process smooth by active sludge treatment method (Margesin *et al.,* 2005). Cold active alkaline pectate lyase isolated from *Becillus sp.* is a good alternative for industrial waste water treatment (Margesin *et al.,* 2005). Bacterial species that are known to produce pectate lyase include *Chryseomonas Luteola* and *Pseudoalteromonas haloplankits* (Laurent *et al.,* 2000; Van *et al.,* 2001). Another important cold adapted enzyme used in bioremediation are lipases which are widely used in waste water treatment and fat degradation in fat contaminated cold environment (Buchon, *et al.,* 2000).

Conclusion

Psychrophilic bacteria are the sources of novel cold-active enzymes of immense biotechnological applications. Efforts have been made worldwide to extract cold adapted enzymes and other products from psychrophilic bacteria. However, the potential of a very small population of such bacteria have been realized so far. The nature offers us a tremendous bacterial diversity for selection and commercial exploration. With the ever increasing pace of bioprospecting studies being undertaken in cold environments worldwide, many unique psychrophilic bacteria are expected to be isolated and identified in future with novel enzymes having great biotechnological applications. The development of metagenomics and PCR techniques have strengthened and redefined the level of our ability to access the enzyme and its gene from any

environment. However, many laboratory scale studies reporting a myriad of psychrophilic bacteria and their cold active enzymes must be scaled up for commercial applications.

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