Applications of Industrially important Cold Active Enzymes: a Mini Review

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Abstract

Psychrophiles, psychrotrophs and psychrotolerant micro-organisms have been considered to be of great importance because of their ability to thrive in cold regions. Their survival in these regions is greatly facilitated by the presence of unsaturated fatty acid layer in their cell membranes, their ability to produce cold shock proteins and cryoprotectants. However, the production of cold active enzymes by these organisms is a fundamental survival strategy of such organisms at such an extreme environment. Recently these enzymes have been considered to be of vital importance mainly because of their commercial purposes for use in different industries at large. These cold active enzymes are thus commercially viable and ecologically constructive as they help in bioremediation in cold regions at low temperatures.

Introduction

Extremophiles as the name suggests, are those microorganisms that can thrive in extremes of environments. They can survive in deep-sea hydrothermal vents, hot springs, polar regions, deep oceans, sulfataric fields and many other extreme environments. Habitats for cold-adapted microorganisms correspond to a large proportion of Earth's area. Polar regions, the montane regions of Europe and several man made habitats of refrigeration and freezer systems contribute to these habitats. The combination of low temperature and low liquid water availability makes these regions extremely uninhabitable for all forms of life. Depending on their growth kinetics cold adapted organisms comprise of two groups psychrophiles and psychrotrophs [1, 2]Also referred to as stenopsychrophile (true psychrophile) and eurypsychrophile(psychrotolerant or psychrotroph) by Feller and Gerday [3].Psychrophiles are defined as organisms that show their optimum growth at about 15°C or lower, a maximal temperature for growth at about 20℃, and a minimal temperature for growth at 0 C or below, Psychrotolerant microbes on the other hand show optimal growth at 20°C or above [2]. These microorganisms mainly belong to the family of bacteria [4 5]archaea, fungi [6] and yeast [7].Cold adapted microorganisms are able to grow at this temperature due to a range of exclusive molecular adaptations like cell membranes, which contain unique lipid constituents to maintain fluidity and enable the transport of substrates, the ability to rapidly synthesize cryoprotectants and cold shock proteins is also a significant factor in microbial survival under low temperature [8-12]. In order to grow at cold temperature the microorganisms need to adapt to the pH and should be resistant to dessication [13]An additional mechanism that these organisms follow are the production of enzymes or biocatalysts that can function at conditions where their mesophilic counterparts fail. Cold active enzymes have an increased structural flexibility which results in reduced activation energies and a consequent increase in their catalytic efficiency [14].The high flexibility and high activity of these psychrophilic enzymes even at low temperatures have been possible because of a decrease in core hydrophobicity, ionic and electrostatic interactions, presence of additional surface loops, presence of more α-helices, but fewer proline residues. Presence of more glycine in surface loops, but decrease in the arginine/lysine ratio, weaker protein interactions and fewer hydrogen bonds and other electrostatic interactions, decreased oligomerization and increase in the number and size of loops and conformational entropy of the unfolded state are some of the factors to name [15, 16].This increases the flexibility between the active site and adjoining regions of cold-active enzymes and also increases the thermolability. Thus the complementarity between the active site and substrate is highly enhanced at a low-energy cost and a high specific activity is achieved even at low temperatures. Thermolability and high activity even at low temperatures are the key features that fulfills the sole significance of psychrophillic enzymes for use in diverse biotechnological and industrial sectors [16-18].The diversity of these microbial enzymes and their distinctive properties like high yields, consistency, immense stability and catalytic activity with economic feasibility, have shown why they are an important choice for use in biotechnology and different industrial areas [19]In addition to being fast and efficient, complete biodegradability [20] and the production of low amounts of by-products is also an added advantage. The application of cold-adapted enzymes has incurred economic benefits through energy savings, removing expensive heating steps, escalating the reaction yields, and reducing adverse chemical reactions even at higher temperatures. Heat input is not required as these enzymes are functionally active even at low temperatures making it a cost efficient enzyme for different industrial processes. Psychrophilic enzymes have become an interesting tool for industrial application as it aids in the efforts to decrease energy consumption. Having a high catalytic activity and low thermal stability even at moderate temperatures, Psychrophiles have modified themselves to suit the extremely cold environments, not found in mesophilic and thermophilic environments. The formation of inclusion bodies are prevented and heat-sensitive gene products are protected at low temperatures. The growth of contaminating microorganisms are limited and the processing times are also shortened. Psychrophilic enzymes can also be utilized in waste-water treatment, biopulping and bioremediation in cold climates [21]. This review gives us a glimpse of the different applications of the three most important microbial cold active enzymes used in the different industries at large and their biotechnological applications.

Alpha amylases:

Microbial enzymes like α-Amylases form some of the most versatile enzymes in the industrial sectors. They have various applications in brewing, baking, pharmaceuticals and textile industries. These enzymes catalyzes the cleavage of the 1,4-α-D-glycosidic linkages in between the adjoining glucose units in starch [22].They are of great importance in the different industries at large, as well as in the biotechnological fields. They have been produced by a large variety of plants, animals and microbes. However the amylases produced by the microorganisms are broadly used in different industries at large, as they are considered to be more stable [23].Besides being stable, the microorganisms are easy to handle and we can control the enzyme and mould it according to our desire. Cold active amylases from psychrophilic, psychrotolerant and psychrotrophic microorganisms are an added advantage as they can function even at cold temperatures and they provide a low activation energy but high activity at these low temperatures that decreases the energy consumption which is a highly beneficial in an era of global energy conservation. They have thus been used at large in different food, detergent and paper industries at large. Bioremediation in cold regions and waste water treatments and its applications in biotechnology have proved its immense ability as a potential enzyme for use in different fields of modern Science.

Proteases

Proteases also referred to as proteinases, or peptidases, cleave the peptide bonds in proteins. Proteases may be of two types intracellular and extracellular. The intracellular proteases maintain the different cellular and metabolic processes whereas the Extracellular proteases hydrolyse the proteins and facilitate the absorption and utilization of hydrolytic products [24].They have long been used in the food and detergent industries. However as the application of cold-active enzymes has increased pace and has become a global phenomenon . The knowledge of cold active protease producing microorganisms and their industrial applications has presently gained momentum. Produced by both prokaryotic and eukaryotic organisms, they have been found to be produced by psychrophilic/psychrotolerant bacteria and fish living in polar regions [25, 26, 3, 12]have reviewed the potentials of cold active organisms and their enzymes. Proteases have been applied in detergent industry, food industry, textile industry,waste management, silver recovery from x-ray films, pharmaceutical industries and different biotechnological applications at large. *Bacillus licheniformis* [27] *Azospirillum* sp. [28] *Colwellia* sp. [29] *Clostridium* sp. [30], *Curtobacteriumluteum* [31], are some of the Cold active protease producing microorganisms isolated from different regions have already found its application in food industries such as baking, cheese processing and breweries. They are also used in leather industry. The cold active protease can be used as additives in detergents that can help in removing stains from fabrics and as the processing can be carried out in low temperature, the colour of clothes will be retained. Cold active protease from B. subtilis was found to be stable in the presence of SDS with enhanced activity in Tween 80 and Wheel detergent which shows that can be potentially used in detergent formulation [32, 33].

Lipases

Lipases are biotechnologically important group of enzymes that catalyze the cleavage of long chain triglycerides. They find immense applications in food, dairy, pharmaceuticals and detergent industries. They are produced by a vast range of bacterial and fungal species. Cold active lipases provide high thermostability and activity even at low temperatures making them the right choice for use in different industrial and biotechnological applications They are particularly of interest in Bioremediation where they can be used to clear oil spills in cold regions like the Antarctic oceans or the Arctic oceans where sea voyages are carried out by the scientists and the researchers at large. At present, lipolytic enzymes are attracting enormous attention due to their adaptable feature and their potential in the field of industry and biotechnology [34] and in a varied numbers of industries like the detergent, food, environmental bioremediation and applications in the biotechnological fields especially while gene cloning where the chances of formation of inclusion bodies in psychrophilic hosts are averted by the use of cold active lipases [25]. Lipases play a significant role in the production of digestive aids [35] emulsifiers [36].According to [37] its varied substrate recognition makes it an important source for use in pharmaceutical applications.

Applications of cold active enzymes in different industries.

Food industries

Processed-food industries that involve baking, brewing, production of fruit juices and starch syrups widely use amylase [38]. These enzymes provide a lot of advantages like : Degradation of the starch in the flour, Enhancement of the rate of fermentation, Improving the taste, crust color and toasting qualities, Provision of anti-staling effect in bread baking, and increasing the shelf life of these products. High specific activity, at low temperatures and easy inactivation are the main keypoints of psychrophilic enzymes. Food processing at higher temperatures elevate chemical reactions and bacterial contaminations which are eliminated by the use of cold active enzymes. The easy inactivation of cold-adapted enzymes by high heat is an added advantage if its inactivation is a cause of concern. Cold-active proteases are widely used in the food industry in making beer, maturing of cheese and baking [39] in the tenderization of meat , taste improvement of refrigerated meat [40]. Soluble protein hydrolysates a form of functional food ingredients are also being produced using proteases. cold-active proteases also find its use in the pet food industry where it is used in the production of digests. In sunflower oil functionalized phenols were esterified using lipases for production of lipophilic antioxidants [41]. According to Feller [3] .The loss of textile fibres due to excessive wear and tear would be highly reduced so will the energy consumption if cold active lipases will be used. The usage of cold active lipases at low temperature will result in the reduction of lesser chemicals, being biodegradable they do not pose an environmental threat. Desizing of denim is one such process in which cold active lipases are used. Cold active lipases have found use in a variety of applications like contact lense cleaner [42], liquid leather cleaner [43]cleaning of exhaust pipes [44]as a bleaching component [45]The cleaning of dirt/ cattle manure [46] etc Lipase has a broad use in food processing with some common use being processing of cheese, flavour improvement, hydrolysis of fat in milk, and addition in alcoholic beverage for enhancing the flavour. Using cold active lipase in food industry can prevent food spoilage and maintain the nutritional value of the heat sensitive substrate that are used in food processing[47-49] Studies have shown also shown that lipase have a potential of synthesizing short chain esters which could be used as flavouring compound in food and pharmaceutical industry[50-52]

Detergent industries

This particular trait is of the utmost importance to the detergent industry. It allows for the development of detergents effective at ambient temperatures that are necessary not only to reduce the environmental and economic impact of reducing washing temperatures but also to supply working products to people with limited access to warm water laundry applications that can be performed at lower temperatures has become feasible because of the application of psychrophilic enzymes in detergents. psychrophilic proteases, amylases or lipases have thus gained great commercial potential for its application in such processes. Amylases, maintain the required stability under detergent conditions as they show activity at lower temperatures and alkaline pH ,oxidative stability on the other hand is an added advantage especially when the washing environment is very oxidizing [53, 54]. Amylase catalyzes the hydrolysis of glucosidic linkages in starch polymers, commonly found in different foods. Using cold active enzymes in these industries reduces energy consumption and being biodegradable is another boon to the environment [55] chemical detergents, high temperatures, vigorous mixing, may seem a promising technique to remove proteinaceous stains, but at the cost of high energy expenditure in the heating step and shortening the life of clothing with lengthy beating. Thus the only alternative here are the cold active enzymes that are active and stable at low temperature and alkaline pH. Thus decreasing the energy consumption and periods of agitation, by cold washing. A lot of work has been done and a lot of patents granted in regard to this aspect. A cold-active serine protease (CP70) produced by *Flavobacterium balustinum* has been patented, that is stable at 30°C for 1 has an alkaline pH and the enzyme activity is not affected by bleaching. Another cold-active alkaline protease showed excellent stability and compatibility with commercial detergents at low temperature It has been isolated from *Stenotrophomonas maltophilia* and shows maximum activity and stability at pH 10 and 20°C. [55]. Washing at lower temperature lowers energy consumption and also decreases the wear and tear of the fabric [48]. These enzymes have a potential to be used as additive in detergent industry which utilizes cold active enzymes. These enzymes helps in reducing the environmental burden as these enzymes are biodegradable and leaves no harmful residues [56] These enzymes can reduce oily stains at the temperature of tap water thus decreasing energy consumption and also the wear and tear of the fabrics. [3]. Surfaces that are infested with molds are also cleaned by cold active lipases in the form of detergents [50, 57]. Fabric processing in textile industry: During the washing process, the textile fibres are often degraded in terms of their smoothness. Adding up cold active lipase can reduce the stress on fabric as they work in low temperature, increasing the softness of fabric. Cold active lipases are thermolabile and can be inactivated easily at higher temperature enhancing the mechanical resistance which helps in improving the final quality of the product.

Biotechnological applications:

Though a lot of proteins may have been produced using Escherichia coli as the host, the expression of eukaryotic proteins in *E. coli* became next to impossible due to formation of protein misfolding and inclusion bodies. However, A cold-active amylase gene from *Alteromonas haloplanctis* has been cloned and expressed.[25, 58]. As such biotechnologically produced or engineered cold active α-amylases could generate avenues for different industrial applications, Site directed mutagenesis has made it possible to produce cold active α-amylases from microorganisms retaining high catalytic activity even at low temperatures. Antarctic psychrophile *Alteromonas haloplanctis* being synthesized at 0±2 °C that can fold correctly when over expressed in Escherichia coli, by the wild strain has also been reported. Over expression of genes in psychrophilic bacteria using a host vector system has also been achieved. A deep-sea psychrotolerant bacterium Pseudoalteromonas sp. SM9913 that produced cold-adapted halophilic proteases has been cloned by Yan et al [76] He cloned and expressed the protease gene as active protein in E. coli BL21 [DE3] cells. Wintrode et al. reversed the properties of a mesophilic subtilisin like protease from Bacillus sphaericus to a protease resembling more to a psychrophilic protease.alkaline protease gene was also cloned from psychrophilic *Planomicrobium*sp. 547 into pTA2 vector Pseudoalteromonas haloplanktis TAC125 was used as a psychrophilic host for recombinant protein production .The cold-active proteases have been used for removal of macromolecular stains from fabrics since the process would be done at low temperature, the colors of the clothes will remain protected on exposure to higher temperature A Cold-active protease from *B. subtilis* has been used in detergent formulation as it showed pH and detergent compatibility at low temperatures Another *Bacillus* sp.158 has found application in contact lens cleaning, One of the application of The protease from *P. aeruginosa* MCM B-327 was found in dehairing hides Cold-active proteases has also substituted rennet to accelerate the ripening of slow-ripening cheeses. cold-active proteases have also been useful in softening and taste development of frozen or refrigerated meat products. Cold-adapted or low temperature tolerant enzymes are very well suited in waste management in cold environments, A new area of research for cost effective production of cold active enzymes has presently come into the limelight. Thus the cloning of genes encoding cold-active protease and their over expression in suitable hosts is the new area of research as of now. Research on cold-active proteases research is wide open .Cloning and expression of serine alkaline protease (SapSh) gene of the psychrotrophic bacterium Shewanella strain Ac10 in E. coli has been reported. [59]. The gene encoding the cold-active protease (pro-2127) from *Pseudoalteromonas* sp QI-1 also has been cloned and expressed [60]. It hasn't been long that the catalytic ability of cold-active proteases at low and moderate temperature and their massive industrial and biotechnological potential has been explored [3, 12, 16, 39, 61, 62, 63] low temperatures prevent the formation of inclusion bodies and protects heat-sensitive gene products, thus protein expression systems capable of operating at low temperatures are the need of the situation in different industries. Although rare, Several researches are being carried out in this regard. A protein expression system has been constructed that allows over expression of genes in psychrophilic bacteria [64].

The degradation potential of a microorganisms in waste is decreased with the decrease in temperature. Organisms producing these cold adapted enzymes can be applied in those cold areas for waste management. According to Buchon et al [65], cold adapted lipases have been used in the field of wastewater treatment, bioremediation of fat in contaminated cold environments, etc [66] investigated the possibility of bioremediation as a treatment option in an alpine glacier area at an altitude of 2,875 m for a chronically diesel-oil-polluted soil and found a significant reduction in the level of one of the most common pollutants, diesel oil, can be achieved even under unfavorable condition.

Paper industries

Polymer-degrading enzymes that are active at lower temperatures are what interests the pulp and paper industries. α-amylases are preferred in the pulp and paper industry for the production of low-viscosity and high molecular weight starch. Starch provides a good coating for the paper , and improves the quality and erasebility. The coating treatment improves the writing quality of the paper by making it soft and smooth. ,As the viscosity of the natural starch is too high for paper sizing the challenge can be overcome by partial degradation with α-amylases in a batch or continuous processes [67]. Being a good sizing agent it enhances the stiffness and strength in paper [68]. Cold active α-amylases are thus preferred for the reduction in the viscosity of starch for suitable coating of paper [69].

Textile industry

Extensive application of cold active proteases are done in the improvement of production methods and fabric finishing. It may be used to remove sericin,without destroying the fibrin making the silk threads more stronger.New and unique finishes are provided by cold active protease treatments by modifying the surface of wool and silk fibres [70]. Proteases from Novozyme (trade name Savinase) sold as an encapsulated detergent is a good example of commercially available protease. Cold-active protease from *Pseudomonas* PL-4 and a cold-active lipase from *Typhiciaishibariensis* have been isolated from cold soil and water by researchers from Hokkaido National Industrial Research Institute (Japan). The Norwegian company Biotec ASA has also been involved in the isolation of cold-adapted enzymes . Enzymes from Antarctica, with a commercial potential has also been a leading area of research for Enzymes in the EU Fourth Framework This could be a promising venture in the expression of heat labile, cold adapted proteins.

Bioremediation

Cold-adapted proteases find its immense application in bioremediation and waste waster treatment. Psychrophilic microorganisms have been anticipated for the bioremediation of wastewaters during the winter in temperate countries, because at low temperatures the degradative capacity of the endogenous microflora is greatly decreased. Cold-adapted microorganisms should be ideal for bioremediation purposes because of the high catalytic efficiency of their enzymes and their unique specificity at low and moderate temperatures [71].

Peptide synthesis

Synthesis of several biologically active peptides has been possible through the use of several microbial proteases[72].Different technologies are being actively explored to overcome the difficulties faced in the enzymatic synthesis of peptides[72]. An indepth understanding of the biological functions and properties of cold active proteases would allow us to develop new methods for the largescale production of biologically active peptides.

Pharmaceutical and agrochemical industry:

Production of optically active chiral compounds was done from the resolution of racemic mixtures of alcohol or carboxylic ester [73]. These chiral intermediates have a high demand in both pharmaceutical as well as agrochemical industry [74]. Production of these optically active chiral compounds using cold active lipase can be much cleaner, ecologically safe and cost effective method.

Application in biosensors:

Use of lipase biosensor has become very popular for diagnostic purposes. Today, these lipase biosensors are widely used for various detection purposes such as analysis of pollution, analysis of adulteration in food industry and pharmaceutical industry. Recently a protease-based biosensor for the detection of schistosome cercariae has been developed [75].

Conclusion:

In the near future, the potential value of cold-adapted enzymes is likely to lead to a greater annual market than for thermostable enzymes. Cold active enzymes from microbes represents a diverse variety of extracellular enzymes produced by them that are proficient enough to carry a wide range of reactions at low temperatures. Thereby providing an interesting field of future research. Extensive screening of new microorganisms for their cold active enzymes capable of functioning at low temperature will pave a novel and simpler route for the synthetic processes. Consequently, this may lay novel ways to solve environmental and biotechnological problems. Much emphasis on the search for novel psychrophilic microorganisms and their cold active enzymes have paved a way for a future where the upcoming industries would incorporate them and thus lead us to an energy efficient world.

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Conflict of Interest

The authors have declared no conflict of interest.

References:

[1] Russell, N. J., (1998). Molecular adaptations in psychrophilic bacteria : potential for biotechnological applications. *Adv.* Biochem. Eng. Biotechnol., **61**, 1-21.

[2] Morita, R.J., (1975). Psychrophilic bacteria. Bacteriol. Rev., **39**, 144-167.

[3] Feller, G., Gerday, C., (2003). Psychrophilic enzymes: Hot Topics in Cold Adaption. Nat. Rev. Microbiol., **1**, 200-208.

[4] Collins, T., Meuwis, M.A., Stals, I., Claeyssens, M., et al., (2002). A novel family 8 xylanase: functional and physico-chemical characterization. J. Biol. Chem ., **277**, 35133–35139.

[5] Okuda, M., Sumitomo, N., Takimura, Y., Ogawa, A., et al., (2004). A new subtilisin family: nucleotide and deduced amino acid sequences of new high-molecular-mass alkaline proteases from *Bacillus* spp. Extremophiles., **8**, 229–235.

[6] Sakamoto, T., Ihara, H., Kozakic, S., Kawasaki, H., (2003). A cold-adapted endo-arabinanase from *Penicillium chrysogenum.* BBA. Gen. Subjects., **1624 (1-3)**, 70–75.

[7] Okamoto, K., Shaw, J.M., (2005). Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu. Rev. Genet., **39**, 503-36.

[8] Finegold, L., (1986). Molecular aspects of adaptation to extreme cold environments. Adv. Space. Res., **6**, 257-264.

[9] Russell NJ (1997) Psychrophilic bacteria—molecular adaptations of membrane lipids. Comp. Biochem. Physiol ., **118**, 489–493

[10] D'Amico, S., Marx, J,C., Gerday, C., Feller, G., (2003). Activity–stability relationships in extremophilic enzymes. J. Biol. Chem., **278**, 7891–7896.

[11] Golovlev, E. L., (2003). Bacterial cold shock response at the level of DNA transcription, translation and chromosome dynamics. Mikrobiologiia., **72**, 5–13.

[12] Georlette, D., Blaise, V., Collins, T., D'Amico, S., et al., (2004). Some like it cold: biocatalysis at low temperatures. FEMS. Microbiol. Rev., **28**, 25–42.

[13] Blaise, G D., Collins, T., D'Amico, S., Gratia, E., et al., (2004). Some like it cold : biocatalysts at low temperatures. *FEMS.* Microbiol. Rev., **1**, 25-42.

[14] Siddiqui, K.S., Cavicchioli, R., (2006). Cold-adapted enzymes. Annu. Rev. Biochem., **75**, 403-433.

[15] Feller, G., (2010). Protein stability and enzyme activity at extreme biological temperatures. J. Phys. Condens. Matter., **22**, 323101–323118.

[16] Cavicchioli, R., Charlton, T., Ertan, H., Mohd Omar, S., et al., (2011). Biotechnological uses of enzymes from psychrophiles. Microb. Biotechnol., **4(4)**, 449-460.

[17] Marx, J.C., Collins, T., D'Amico, S., Feller, G., et al., (2007). Cold-adapted enzymes from marine Antarctic microorganisms. Mar. Biotechnol (NY)., **9**, 293–304.

[18] Feller, G., (2013). Psychrophilic enzymes: from folding to function and biotechnology. Scientifica (Cairo)., **2013**, 512840.

[19] Gurung, N., Ray, S., Bose, S., and Rai, V., (2013). A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. Biomed. Res. Int., **2013**, 329121.

[20] Rozzell, J. D., (1999). Commercial scale biocatalysis: myths and realities. Bioorg. Med. Chem., **7**, 2253–2261.

[21] Kuddus, M., Ramteke, P.W., (2009). Cold-active extracellular alkaline protease from an alkaliphilic *Stenotrophomonas maltophilia*: production of enzyme and its industrial applications. Can. J. Microbiol., **55**, 1294–1301.

[22] Prakash, B., Vidyasagar, M., Madhukumar, M.S., Muralikrishna, G., et al., (2009). Production, purification, and characterization of two extremely halotolerant, thermostable, and alkali-stable -amylases from *Chromohalobacter* sp. TVSP. Process. Biochem., **44**, 210–215.

[23]Tanyildizi, M.S., Ozer, D., Elibol, M., (2005). Optimization of -amylase production by *Bacillus* sp. using response surface methodology. Process. Biochem ., **40**, 2291–2296.

[24] Hartley, B.S., (1960). Proteolytic enzymes. Annu. Rev. Biochem., **29**, 45-72.

[25] Feller, G., Narinx, E., Arpigny, J.L., Aittaleb, M., et al., (1996). Enzymes from Psychrophilic Organisms. FEMS. Microbiol. Rev., **18**, 189 -202.

[26] Margesin, R., Feller, G., Gerday,C., Russell, N., (2002). Cold-Adapted micro-organisms: adaptation strategies and biotechnological potential. In: Bitton G. (ed.). The Encyclopedia of Environmental Microbiology, Vol. 2. New York: John Wiley & Sons, pp. 871–885.

[27] Baghel, V.S., Tripathi, R.D., Ramteke, R.W., Gopal, K., et al.,(2005). Psychrotrophic proteolytic bacteria from cold environments of Gangotri glacier, Western Himalaya India . Enzyme. Microbial. Technol., **36**, 654-659.

[28] Oh, K.H., Seong, C.S., Lee, S.W., Kwon, O.S., et al., (1999).Isolation of a psychrotrophic *Azospirillum* sp. and characterization of its extracellular protease. FEMS. Microbiol. Lett., **174**, 173-178.

[29] Wang, Q., Hou, Y., Xu, Z.,Miao, J., et al., (2008). Optimization of cold-active protease production by the psychrophilic bacterium *Colwellia* sp NJ341 with response surface methodology. Biores. Technol., **99**, 1926-1931.

[30] Alam, S.I., Dube, S., Reddy, G.S.N., Bhattacharya, B.K., et al., (2005). Purification and characterization of extracellular protease produced by *Clostridium* sp. from Schirmacher oasis, Antarctica. Enzyme. Microbial. Technol., **36**, 824-831.

[31] Kuddus, M., Ramteke, P.W., (2008). A cold�active extracellular metalloprotease from *Curtobacterium luteum*. (MTCC 7529), enzyme production and characterization. J. Gen. Appl. Microbiol., **54**, 385-392.

[32] Joshi, Swati, et al., (2012). Characteristics and applications of a recombinant alkaline serine protease from a novel bacterium *Bacillus lehensis*. Bioresource. Technol., **131**, 76 – 85.

[33] Baghel, V.S.,Tripathi, R.D., Ramteke, R.W., Gopal, K., et al., (2005).Psychrotrophic proteolytic bacteria from cold environments of Gangotri glacier, Western Himalaya India. Enzyme. Microbial. Technol., **36**, 654-659.

[34] Jaeger, K.E., Eggert, T., (2002). Lipases for biotechnology. Curr. Opin. Biotechnol., **13(4)**, 390–397.

[35] Vulfson, E.N., (1994). Industrial applications of lipases. In: P Wooley, SB Petersen (eds) Lipases, Cambridge, Great Britain: Cambridge University Press, p. 271.

[36] Sharma, R., Chisti, Y., Banerjee, U.C., (2001). Production, purification, characterization and applications of lipases. Biotechnol. Adv., **19**, 627-662.

[37] Margesin, R., Feller, G., Gerday, C., Rusell, N., (2002). Cold adapted Microorganisms: Adaptation strategies and Biotechnological Potential. In: Bitton (ed). The Encyclopedia of Environmental Microbiology, John Wiley & Sons, New York, pp. 871-885.

[38] Zeeman, N., McCrea, J., (1985). Alpha-amylase Production Using a Recombinant DNA Organism. Cereal Food World., **30**, 777-780.

[39] Margesin, R., Schinner, F., (1994). Properties of cold adapted microorganisms and their potential role in biotechnology. J. Biotechnol., **33**, 1–14.

[40] Orange, N., (1994). Growth temperature regulates the induction of beta-lactamase in *Pseudomonas fluorescens* through modulation of the outer membrane permeation of a betalactam- inducing antibiotic. Microbiology (Reading, Engl), **140** (Pt 11), 3125–3130.

[41] Buisman, G.J.H., Helteren, C.T.W., Kramer, G.F.H., Veldsink, J.W., Derksen, J.T.P., et al., (1998). Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol. Lett., **20**, 131-136.

[42] Bhatia, R,P., (1990). Contact lens cleaning composition containing an enzyme and a carboxylvinyl polymer. United States Patent., **4**,921,630.

[43] Kobayashi, H., (1989). Liquid leather cleaners. Japanese Patent 1,225,700.

[44] Moriguchi, H., Hirata, J., Watanabe, T., (1990). Microorganism based agent for treatment of organic wastes. Japanese Patent., 2,105,899.

[45] Nakamura, K., Nasu, T., (1990). Enzyme containing bleaching composition, Japanese Patent 2, 208,400.

[46] Abo, M., (1990). Method of purifying dry-cleaning solvent by decomposing liquid contaminants with a lipase. World Organization Patent 9,007,606.

[47] Cavicchioli, R., Siddiqui, K.S., Andrews D., Sowers, K.R., (2002). Low temperature extremophiles and their applications. Curr. Opin. Biotechnol., **13**, 253–261.

[48] Gerday, C., Aittaleb, M., Bentahir, M., et al ., (2000). Cold-adapted enzymes: from fundamentals to biotechnology. Trends. Biotechnol., **18**, 103–107.

[49] Russell, N.J., (1998). Molecular adaptations in psychrophilic bacteria : potential for biotechnological applications. Adv. Biochem. Eng. Biotechnol., **61**, 1-21.

[50] Malangwa, J., Ali, M.S.M., Salleh, A.B., Abd Rahman, R.N.Z.R., et al., (2014). Adaptational properties and applications of cold-active lipases from psychrophilic bacteria. Springer Japan., **19**, 235–247.

[51] Li, M., Yang, L-R., Xu, G., Wu, J.P., (2013). Screening, purification and characterization of a novel cold-active and organic solvent-tolerant lipase from *Stenotrophomonas maltophilia* CGMCC 4254. Bioresour. Technol., **148**, 114–120.

[52] Brault, G., Shareck, F., Hurtubise, Y., et al., (2012). Isolation and characterization of EstC, a new cold-active esterase from *Streptomyces coelicolor* A3(2). PLoS ONE, **7**, e32041.

[53] Kuddus, M., (2014). Bio-statistical approach for optimization of cold-active α-amylase production by novel psychrotolerant *M. foliorum* GA2 in solid state fermentation. Biocat. Agri. Biotech., **3(2)**, 175-181.

[54] Kirk, O., Borchert, T.V., Fuglsang, C.C., (2002). Industrial enzyme applications. Curr. Opin. Biotechnol., **13**, 345-351.

[55] Kuddus, M., Ramteke, P.W., (2009). Cold-active extracellular alkaline protease from an alkaliphilic *Stenotrophomonas maltophilia*: production of enzyme and its industrial applications. Can. J. Microbiol ., **55**, 1294–1301.

[56] Joseph, B., Ramteke, PW., Thomas, G., (2008). Cold active microbial lipases ; Some hot issues and recent developments. Biotechnol. Adv., **26**, 457–470.

[57]Valentini, F., Diamanti, A., and Palleschi, G., (2010). New bio-cleaning strategies on porous building materials affected by biodeterioration event. Appl. Surf. Sci., **256**, 6550–6563.

[58] Feller, G., Lonhienne, T., Deroanne, C., Libioulle, C., et al., (1992). Purification, characterization, and nucleotide sequence of the thermolabile α-amylase from the Antarctic psychrotroph *Alteromonas haloplanctis* A23. J. Biol. Chem., **267**, 5217-522.

[59] Kulakova, L., Galkin, A., Kurihara, T., Yoshimura, T., et al., (1999). Cold active serine alkaline protease from the psychrotrophic bacteria Shewanella strain Ac10: gene cloning and enzyme purification and characterization. Appl. Environ. Microbiol., **65**, 611–617.

[60] Guoying, X., Shuoshuo, C., Xuezheng L.,et al., (2011). Cloning and heterologous expression of pro-2127, a gene encoding cold-active protease from *Pseudoalteromonas* sp. QI-1. Adv. Polar. Sci., **22**, 124–130.

[61] Demirjian, D.C., Morís-Varas, F., Cassidy, C.S., (2001). Enzymes from extremophiles. Curr. Opin. Chem. Biol., **5**, 144–151.

[62] Burg, B.V., (2003). Extremophiles as a source for novel enzymes. Curr. Opin. Microbiol., **6**, 213–218.

[63] Gupta, R., Beg, Q.K., Lorenz, P., (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol., **59**, 15–32.

[64] Tutino, M, L., (2001). A novel replication element from an Antarctic plasmid as a tool for the expression of proteins at low temperature. Extremophiles., **5**, 257–264.

[65] Buchon, L., Laurent, P., Gounot, A.M., Guespin, M.J.F., (2000). Temperature dependence of extracellular enzyme production by psychotrophic and psychrophilic bacteria. Biotechnol. Lett., **22**, 1577-1581.

[66] Margesin, R., Dieplinger, H., Hofmann, J., Sarg, B., et al., (2005).A cold-active extracellular
metalloprotease from *Pedobactercryoconitis*-production and properties. Res. Microbiol., **156**, 499-505.

[67]Fryer, P.J., Asteriadou, K.A., (2009). Prototype cleaning map: a classification of industrial cleaning processes. Trends. Food. Sci. Tech., **20**, 255-262.

[68] Bruinenberg, P.M., Hulst, A.C., Faber, A., Voogd, R. H ., (1996). A process for surface sizing or coating of paper. European Patent Application., EP 0 690 170 A1.

[69] Kuddus, M., Roohi, (2010). Microbial Cold-Active α-Amylases: From Fundamentals to Recent Developments. In: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Vilas, M.A. (Ed.). Vol. 2, Formatex Research Center, Spain, pp. 1265-1276.

[70] Najafi, M.F., Deobagkar, D., Deobagkar, D., (2005). Potential application of protease isolated from *Pseudomonas aeruginosa* PD100. Electron. J. Biotechnol., **8**, 197-203.

[71] Margesin, R., Schinner, F., (1999). Biological decontamination of oil spills in cold environments (review). J. Chem. Technol. Biot., **74**, 1-9.

[72] Kumar, D., Bhalla, T.C., (2005). Microbial proteases in peptide synthesis: approaches and applications. Appl. Microbiol. Biotechnol, **68**, 726–736.

[73] Akita, H., Chen, C.Y., Uchida, K.A., (1995). Purification and characterization of heat stable lipases from *Pseudomonas fluorescence*. Tetrahedron : Asymmetry., **6**, 2131-2134.

[74] Patel, R.N., (2002). Microbial/enzymatic synthesis of chiral intermediates for pharmaceuticals. Enzyme. Microb. Technol., **31**, 804–826.

[75] Webb, A.J., Kelwick , R. Doenhoff, M.J., Kylilis, N., et al., (2016). A protease-based biosensor for the detection of *schistosome cercariae*. Sci. Rep., **6**, 24725.

[76] Yan, B.Q., Chen, X.L., Hou, X.Y., et al., (2009). Molecular analysis of the gene encoding a cold-adapted halophilic subtilase from deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913: cloning, expression, characterization and function analysis of the C-terminal PPC domains. Extremophiles., **13**, 725–73.