International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Issue 1; January 2018; Page No. 340-345

Cold-active alkophilic proteases from various microbial sources: benefits and applications

Junaid Furhan, * Dr. Sarika Sharma
Department of Biotechnology and Microbiology, Arni School of Basic Sciences, Arni University, Indora, Himachal Pradesh, India

Abstract
From immeasurable time, microorganisms evolved and accumulated everywhere despite of variations in pH and temperature. Remarkable physiological and functional heterogeneity of microorganisms make them major resource for bioprospection of industrial enzymes. This review describes the cold active alkophilic proteases that can work in extreme conditions of low temperature and high alkalinity, produced by a wide range of psychrophilic and psychrotrophic microorganisms. Different biochemical properties and cloning strategies affecting the production of cold active alkophilic proteases are discussed which enable selection of promising organisms for industrial production, and exploration of cold active alkophilic proteases in diverse industrial applications is highlighted.

Keywords: alkophilic, psychrophilic, cold active, protease

Introduction

Microbes include an immense range of organisms that are found almost everywhere on earth. In the midst of these microorganisms some of them are competent to grow in odd and inconsiderate environmental conditions such as excess salinity, hot springs, low temperature levels, high pressures, or extreme pH (Fujiwara 2002) [11]. According to Cavicchioli *et al*. (Cavicchioli *et al*. 2002) [4], the majority of microbes that thrive in low temperature environments are psychrophiles or psychrotrophs (psychrotolerants). Additionally, these workers differentiated psychrophiles from psychrotrophs (psychrotolerants) according to their optimal temperature, suggesting that psychrophilic organisms have maximum growth at a temperature of ≤ 15°C, however incapable of growing above 20°C whereas latter have utmost growth at temperature ≥ 20°C, but can show growth at temperatures close to 0°C. Gomes *et al*. (Gomes and Steiner 2004) defined those microorganisms that necessitate extreme environments for growths are extremophiles and the enzymes produced by them are extremozymes.

Despite the fact that psychrophiles are present in low temperature environments, their proteins and membranes are integrated with distinctive features and mechanisms that help them in maintaining metabolic rates at low temperatures. They have successfully found way to cope with low temperatures by synthesizing enzymes that facilitate their survival (Feller and Gerday 2003) [10]. These enzymes having high levels of activity and thermolability at low temperatures are generally termed as cold active enzymes or extremozymes. Present biotech industry is in need of such extremozymes that can function under extreme conditions and potentially can be involved in various industrial applications. These extremozymes can replace the existing enzymes that require high temperature for performance (Marchi *et al*. 2007; Margesin 1999) [30, 31]. The factors responsible for high catalytic efficiency of these cold-active enzymes are high flexibility and higher turnover number (kcat) at the expense of Km or by optimizing both parameters (Morgan-Kiss *et al*. 2006) [38]. Although different kinds of cold-active enzymes have been isolated from cold adapted microorganisms, still proteases are considered as the most vital group of hydrolytic enzymes having high catalytic efficiencies at lower temperatures (Rao *et al*. 1998) [41, 42]. Currently, competition is increasing within the enzyme market with new technologies and advancements. Proteases are been used increasingly in various industries and these proteases seize two-third of the total enzyme market share worldwide (Kumar and Takagi 1999) [28] and over the past few years, microbial cold-active proteases have been acknowledged that present massive potential for biotechnological industries. The psychrophilic and alkophilic nature of proteases has made them a first choice candidate in detergent industry particularly with many of the products been successfully launched at commercial level by many leading companies (Alam *et al*. 2005; Cavicchioli *et al*. 2002; Demirjian *et al*. 2001; Eichler 2001; Feller and Gerday 2003; Gerday *et al*. 2000; Margesin *et al*. 2002; Margesin and Schinner 1994; Van Den Burg 2003; Zeng *et al*. 2003) [1, 4, 7, 9, 10, 34, 35, 13, 49, 52, 33]. According to a newly published report from Business Communications Company, the global industrial enzyme market has reached nearly $4.6 billion and $4.9 billion in 2014 and 2015, respectively. It's presumed that this market will increase from nearly $5.0 billion in 2016 to $6.3 billion in 2021 at a compound annual growth rate (CAGR) of 4.7% for 2016-2021. The detergent industrial enzyme mark*et al*one is expected to grow from $1 billion in 2016 to nearly $1.4 billion in 2021 (Dewan S 2017) [8].

The comprehensive investigations on cold-active proteases and their adaptation to cold environments along with their structure and bioenergetics are sparse. Modest information is available on enzymes from microorganisms isolated from glacier regions that may be ideal habitat for cold adapted

organisms. The application potential of such proteases with unique qualities needs to be exploited fully for the benefit of mankind. This review highlights the cold active alkophilic proteases from microbial sources with high potential in the low temperatures and alkaline environments.

Microbial sources and habitat of cold-active alkophilic proteases

Through incessant exploration of environments with low temperatures, psychrophiles of more than 100 species have been identified and reported so far. Psychrophiles acquire the ability of degrading a wide range of polymeric substances by producing their respective enzymes (Cavicchioli *et al*. 2002; Deming 2002; Demirjian *et al*. 2001; Georlette *et al*. 2004; Gerday *et al*. 2000; Margesin *et al*. 2002; Van Den Burg 2003) [4, 6, 7, 12, 13 34, 49]. Cold-active alkophilic protease producing microorganisms have been isolated and reported from different geographical regions, such as a marine bacterium, *Alteromonas haloplanktis* produced a thiol protease that has shown activity in low temperature conditions (Suzuki and Odagami 1997) [47]. Moderately halotolerant, SDS stable alkophilic protease has been produced from *Bacillus cereus* MTCC 6840, reported from lake Nainital, Uttaranchal state, India (Joshi *et al*. 2007) [21]. Kuddus *et al*. reported a novel psychro-tolerant bacterium, *Curtobacterium luteum* from the soil of Gangotri glacier, Western Himalaya, secreted an extracellular protease at low temperature (Kuddus and Ramteke 2008b) [24]. Psychrotolerant proteolytic bacteria *S. Maltophilia* MTCC 7528 from soil of Gangotri glacier, Western Himalaya, India, produced cold-active extracellular alkophilic protease that may be a prospective component to be used as a detergent additive for cold washing that will be beneficial to save energy as they work at lower temperatures (Kuddus and Ramteke 2011) [25]. An extracellular protease producing bacteria, *Pseudoalteromonas* sp. has been isolated from King George Island, Antarctica. The cold active protease produced by the strain can be a probable candidate for industrial applications (Vázquez *et al*. 2008) [50]. Newly produced cysteine protease (SpCP) from a psychrotolerant strain, *Serratia proteamaculans* 94 showed activity in low temperatures (Mozhina *et al*. 2008) [39]. Psychrotrophic *Shewanella* species exhibited a high level of protease activity at low temperatures and a subtilisin like protease gene has been cloned from this bacterium (Kulakova *et al*. 1999) [27]. A novel species *Sporosarcina macmurdoensis* from McMurdo Dry Valleys, Antarctica, was reported to be psychrophilic and alkophilic as well (Reddy *et al*. 2003) [43]. Psychrotolerant bacterium, *Stenotrophomonas* sp. producing alkophilic protease has been reported from Thajiwas glacier of Kashmir, India (Saba *et al*. 2012) [45]. A psychrophilic strain, *Colwellia psychrerythraea* 34H produced an extracellular cold-active aminopeptidase (Huston *et al*. 2004) [19]. A deep-sea psychrophilic bacterium, *Pseudomonas* sp. DY-A produced an extracellular cold-active and alkophilic protease (Zeng *et al*. 2003) [52]. Extracellular cold-active protease producing bacterium, *Pedobacter cryoconitis* was reported from alpine cryoconite on glacier ice (Margesin *et al*. 2005) [32]. An anaerobic, psychrotrophic bacterium, *Clostridium* sp. from Schirmacher oasis, Antarctica, produced extracellular cold-active protease (Alam *et al*. 2005) [1]. Table 1 below shows microorganisms that strictly follow the definition of cold-active alkophilic proteases**.**

Table 1: Microorganism known to produce cold-active alkophilic proteases

S. No.	Sources of cold active alkophilic proteases	Protease type	Mol. Wt. (kDa)	Optimum Temp.	Optimum pH	Reference
1.	*Alteromonas haloplanktis*	Thiol protease	74-76	20	8-9	(Suzuki and Odagami 1997) [47]
2.	*Bacillus cereus*	Metal-activated enzyme	n.s	20	9	(Joshi *et al*. 2007) [21]
3.	*Clostridium* sp.	Serine-type Metalloenzyme	n.s	10-20	8	(Alam *et al*. 2005) [1]
4.	*Colwellia psychrerythraea strain 34H*	Aminopeptidase	71	19	6─8.5	(Huston *et al*. 2004) [19]
5.	*Curtobacterium luteum*	Metalloprotease	115	20	7	(Kuddus and Ramteke 2008b) [24]
6.	*Pedobacter cryoconitis*	Metalloprotease	27	15	7	(Margesin *et al*. 2005) [32]
7.	*Pseudoalteromonas* sp. P96-47	Metalloprotease	n.s	20	8	(Vázquez *et al*. 2008) [50]
8.	*Serratia proteamaculans* 94	Cysteine protease	50	4─30	8	(Mozhina *et al*. 2008) [39]
9.	*Shewanella* strain Ac10	Alkaline serine protease	44	5─15	9	(Kulakova *et al*. 1999) [27]
10.	*Sporosarcina macmurdoensis*	n.s	n.s	18─20	7	(Reddy *et al*. 2003) [43]
11.	*Stenotrophomonas* sp.	Alkaline protease	55	15	10	(Saba *et al*. 2012) [45]
12.	*Stenotrophomonas maltophilia* MTCC 7528	Alkaline protease	75	20	10	(Kuddus and Ramteke 2011) [25]

n.s = not specified

Biochemical properties of cold active alkophilic proteases

pH

Mostly alkophilic micro-organisms strongly depend on the presence of extracellular pH required for cell growth and enzyme production. The pH of the reaction mixture greatly defines the activity of any enzyme. Proteases differ in their classes due to difference in their pH optima. Protease yields from alkophiles can be increased by maintaining the pH of the medium above 7.5 during the fermentation period (Aunstrup 1980) [2].

Transportation of various components across the cell membrane and many enzymatic processes are affected strongly affected by the culture pH (Moon and Parulekar 1991) [37].

The pH of cold-active alkophilic protease producing organisms differs greatly, and the highest pH optima has been reported at pH 10 (Kuddus and Ramteke 2011; Saba *et al*. 2012) [25, 45].

Temperature and Thermostability

Temperature is one of the critical parameters that vary from one microorganism to another. The mechanism of temperature control has not been fully understood for the enzyme production so it needs to be carried in a controlled manner (Chaloupka 1984) [5]. However, cold active proteases used in industrial applications can be engineered by improving their specific properties. Enzyme engineering can help to enhance inherent properties that's includes increased thermolability/or catalytic activity at low temperatures. A thermostable enzyme that retains high catalytic activity can find application as cold active enzyme in high temperature processes. These types of thermostable enzymes can be produced using site-directed mutagenesis (Cavicchioli *et al*. 2002) [4]. Alkophilic proteases produced from *Bacillus* sp., *Streptomyces* sp. and *Thermus* sp. have shown further enhanced enzyme thermostability after the addition of Ca^{2+} (Kumar and Takagi 1999) [28].

Effect of metal ions, inhibitors and detergents

Metal ions that are divalent in nature such as boron, cobalt, calcium, copper, magnesium, manganese, and molybdenum can be used for optimum production of alkophilic protease in the fermentation medium. The source of enzyme decides the requirement for special metal ions. (Kumar and Takagi 1999) [28]. Metal ions such as Fe^{++} and Co^{++} have been reported to enhance the activity of protease produced from *Bacillus cereus* MTCC 6840. However the alkaline protease showed negative effect in the presence of Ca^{++}, Cu^{++}, K^{+}, Mg^{++}, Mn^{++}, $NH4^{+}$ and was stable in the presence of Na^{+} (Joshi *et al*. 2007) [21]. Extracellular cold-active protease from *Clostridium* sp. was inhibited by EDTA, PMSF, Zn^{2+} and iodoacetamide but the protease remained defiant to SDS detergent (Alam *et al*. 2005) [1]. *Colwellia psychrerythraea* Strain 34H cold-active aminopeptidase was strongly inhibited by a metal chelating agent (EDTA) along with divalent cations, Zn^{2+} and Mn^{2+}. The protease activity was slightly inhibited in the presence of Ca^{2+} and enhanced in the presence of Mg^{2+} (10 mM) (Huston *et al*. 2004) [19]. *C. luteum* protease activity was strongly inhibited by metal ions such as Cu^{2+}, Ca^{2+}, K^{+}, Ba^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+}, and Mg^{2+}, whereas the activity enhanced by 35% in presence of Mn^{2+}. The protease was classified as metalloprotease due to the fair inhibition caused by EDTA (Kuddus and Ramteke 2008a) [23]. *Pedobacter cryoconitis* protease was sensitive towards EDTA, EGTA, Sodium perborate and SDS and phenanthroline. The protease was classified as metalloprotease (Margesin *et al*. 2005) [32]. Vazquez *et al*. reported that proteases from *Pseudoalteromonas* sp. P96-47 were inhibited by phenanthroline and EDTA but the activity wasn't affected by PMSF, indicating that the protease belongs to the group of metalloproteases. However, the protease activity was fairly affected by metal ions like Zn^{2+}, Hg^{2+}, Cu^{2+} and Ni^{2+} (Vázquez *et al*. 2008) [50]. *S. proteamaculans* protease was classified as cysteine protease as the enzyme activity was entirely inhibited by $HgCl_2$, p-chloromercury benzoate and Chymostatin. The activity of the protease stimulated in the presence of Dithiothreitol, Cysteine and Glycyl-glycine (Mozhina *et al*. 2008) [39]. *Stenotrophomonas maltophilia* MTCC 7528 protease was partially inhibited by presence of heavy metals such as Hg^{2+}, Cd^{2+}, Zn^{2+} and Co^{2+} while the activity was enhanced by Cu^{2+} and Cr^{2+} (Kuddus and Ramteke 2011) [25].

Cloning

Recombinant DNA technology (rDNA) is used for microbial stain improvement by generating selective mutants that exhibit higher protease production. It's a rapidly progressing technology that has opened up new opportunities for manipulation and control of genes. Cloning of the gene encoding an alkophilic protease marks an essential step in the engineering of most efficient producer microorganisms with *E.coli* and *B.subtitlis* being the two microorganism of choice (Gupta *et al*. 2002). This method helps us to understand the structural and functional relationship of genetic systems (Rao MB 1998) [41, 42]. With new rDNA techniques above 50% of the enzymes used in various industries are now produced from genetically engineered microorganisms (Hodgson 1994) [17]. In case of psychrophiles, scientists have made very few attempts in cloning and expression of cold-active proteases either in heterologous or homologous hosts (Joshi and Satyanarayana 2013) [22]. A cold adapted halophilic protease has been cloned from deep sea psychrotolerant bacterium *Pseudolteromonas* sp SM9913. Protease gene was cloned into pET22b (+) and the gene was expressed in *E.coli* BL21 [DE3] cells as an active protein. Further a multidomain recombinant protein was purified from fermentation broth consisting of catalytic domain and two PPC domains that were further characterized using purified recombinant protein (Yan *et al*. 2009) [51]. Psychrophilic features of a cold-active protease were improved by cloning and expression technology. The k_{cat}/k_m value of mutant subtilisin m-63 improved 100% than that of wild type at 10°C. The expression vector pUC18 was used for *E.coli* and pFY300PLK was used for *Bacillus* and *E.coli* JM109 and *Bacillus subtilis* UOT0999 as expression vectors respectively. It was observed that enhanced substrate affinity was mostly responsible for increase in activity (Taguchi *et al*. 1998) [48]. *Pseudoalteromonas haloplanktis* TAC125 has been developed as a versatile psychrophilic host for production of recombinant protein by disrupting its *gsp*E gene (Parrilli *et al*. 2008) [40]. More psychrophilic hosts need to be generated so that a wide range of cold-active proteins along with industrially important protease can be heterologously expressed.

Applications of cold-active alkophilic proteases

Cold-adapted proteases have a propensity to have high biotechnological value due to their high k_{cat} at temperatures ranging from low to moderate. They possess high thermolability at elevated temperatures and also have the ability to function in organic solvents for the purpose of organic synthesis (Cavicchioli *et al*. 2002; Gerday *et al*. 2000; Margesin and Feller 2010; Marx *et al*. 2007; Siddiqui and Cavicchioli 2006) [4, 13, 46, 36, 33].

Cold-adapted proteases can be an economic boost by being more productive than mesophilic or thermophilic homologues at low temperature, thereby providing energy savings to the

processes that the proteases are used in. As a result, cold-adapted proteases have found application in industries as diverse as household detergents, baking industrial functions, textiles, cleaning/hygiene products, molecular biology, environmental bioremediations (reducing contamination), consumer food products (dairy manufacturing and preparation), cosmetics, and pharmaceuticals (as biocatalysts in organic synthesis of drugs and/or intermediates in their generation) (Huston 2008; Kuddus and Ramteke 2012) [18, 26].

Use of cold-adapted proteases allows reducing the undesirable chemical reactions that can occur at higher temperatures, the proteases can be rapidly inactivated by heating, and they can be used to transform substrates that require enzyme reactions to be performed at low temperature because substrates are heat-sensitive (Jeon *et al*. 2009) [20]. Such properties of cold active proteases find importance in food and feed industry to avoid spoilage, alteration in nutritional value and flavor of the original heat-sensitive substrates and products (Cavicchioli *et al*. 2002; Gerday *et al*. 2000; Luisa Tutino *et al*. 2009; Russell *et al*. 1998) [4, 13, 29, 44].

Therapeutically proteases have been used in four areas: the management of gastrointestinal disorders with orally administered agents, as anti-inflammatory agents, as thrombolytic agents for thromboembolic disorders, and as locally administered agents for wound debridement (Gudmundsdóttir and Pálsdóttir 2005) [15].

Cold-adapted proteases can establish well within the waste management in cold environments, where the degradation capabilities of endogenous micro-flora are reduced due to low temperatures. Cold-adapted proteases thus can be used to optimize present day industrial processes and for developing future technologies with less energy inputs and process cost by removing the cost of heat inactivation step (Cavicchioli *et al*. 2002; Margesin *et al*. 2002) [4, 34].

Despite their biotechnological potential, and in comparison with the use of thermostable proteases, few cold-adapted proteases are in commercial use. Examples of commercial applications include a protease from Novozyme (trade name Savinase) sold as an encapsulated detergent (Bull *et al*. 1998) [3].

Conclusion

Microorganisms from diverse habitats, permanently cold as well as those exposed to cold are termed as psychrophilic or psychrotrophic and are known to produce cold-active enzymes, psychrophilic enzymes, or extremozymes. Such enzymes carry great importance in the present era and they find large applications on industrial scale. Psychrophiles with such enzymes have been exploited within limits and have expanded only in recent years. Scientists are working to tailor new enzymes that are active at low temperatures and also can thrive in alkophilic conditions making them suitable for biotechnological applications. Keeping the future aspects in view, cold environments call for expanded exploration so as to discover and characterize new strains and evaluate their biotechnological potential. The field of cold-active protease research is still wide open and expected to achieve extravagant achievement in the nearest future.

Acknowledgements

The authors acknowledged with thank for generous support of the Arni University and Department of Biotechnology for providing the facilities to carry out the work.

Ethical Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Alam S, Dube S, Reddy G, Bhattacharya B, Shivaji S, Singh L. Purification and characterisation of extracellular protease produced by Clostridium sp. from Schirmacher oasis, Antarctica. Enzyme and Microbial Technology. 2005; 36(5):824-831.
2. Aunstrup K. Proteinases in Economic Microbiology: Microbial Enzymes and Bioconversions (AH Rose ed.), Academic Press, New York. 1980, 5:49-114.
3. Bull A, Marrs B, Kurane R. Biotechnology for clean industrial products and processes. Towards industrial sustainability. OECD Publications. 1998, 7-139.
4. Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR. Low-temperature extremophiles and their applications. Current Opinion in Biotechnology. 2002; 13(3):253-261.
5. Chaloupka J. Temperature as a factor regulating the synthesis of microbial enzymes. Microbiological sciences. 1984; 2(3):86-90.
6. Deming JW. Psychrophiles and polar regions. Current opinion in microbiology. 2002; 5(3):301-309.
7. Demirjian DC, Morís-Varas F, Cassidy CS. Enzymes from extremophiles. Current opinion in chemical biology. 2001; 5(2):144-151.
8. Dewan S SK. Global markets for enzymes in industrial applications. Wellesley, MA: BCC Reseaerch. 2017.
9. Eichler J. Biotechnological uses of archaeal extremozymes. Biotechnology advances. 2001; 19(4):261-278
10. Feller G, Gerday C. Psychrophilic enzymes: hot topics in cold adaptation. Nature reviews microbiology. 2003; 1(3):200-208.
11. Fujiwara S. Extremophiles: Developments of their special functions and potential resources. Journal of bioscience and bioengineering. 2002; 94(6):518-525.
12. Georlette D, Blaise V, Collins T, D'Amico S, Gratia E, Hoyoux A. Some like it cold: biocatalysis at low temperatures. FEMS microbiology reviews. 2004; 28(1):25-42.
13. Gerday C, Aittaleb M, Bentahir M, Chessa J-P, Claverie P, Collins T. Cold-adapted enzymes: from fundamentals to biotechnology. Trends in biotechnology. 2000; 18(3):103-107.
14. Gomes J, Steiner W. The biocatalytic potential of extremophiles and extremozymes. Food technology and Biotechnology. 2004; 42(4):223-235.
15. Gudmundsdóttir Á, Pálsdóttir HM. Atlantic cod trypsins: from basic research to practical applications. Marine biotechnology. 2005; 7(2):77-88.
16. Gupta R, Beg Q, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. Applied microbiology and biotechnology. 2002; 59(1):15-32.
17. Hodgson J. The changing bulk biocatalyst market. Nature Biotechnology. 1994; 12(8):789-790.

18. Huston AL. Biotechnological aspects of cold-adapted enzymes. Psychrophiles: from biodiversity to biotechnology. 2008, 347-363.
19. Huston AL, Methe B, Deming JW. Purification, characterization, and sequencing of an extracellular cold-active aminopeptidase produced by marine psychrophile Colwellia psychrerythraea strain 34H. Applied and environmental microbiology. 2004; 70(6):3321-3328.
20. Jeon JH, Kim J-T, Kim YJ, Kim H-K, Lee HS, Kang SG. Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. Applied microbiology and biotechnology. 2009; 81(5):865-874.
21. Joshi GK, Kumar S, Sharma V. Production of moderately halotolerant, SDS stable alkaline protease from Bacillus cereus MTCC 6840 isolated from lake Nainital, Uttaranchal state, India. Brazilian Journal of Microbiology. 2007; 38(4):773-779.
22. Joshi S, Satyanarayana T. Biotechnology of cold-active proteases. Biology. 2013; 2(2):755-783.
23. Kuddus M, Ramteke PW. A cold-active extracellular metalloprotease from Curtobacterium luteum (MTCC 7529): enzyme production and characterization. The Journal of General and Applied Microbiology. 2008a; 54(6):385-392.
24. Kuddus M, Ramteke PW. Purification and properties of cold-active metalloprotease from Curtobacterium luteum and effect of culture conditions on production. Chinese Journal of Biotechnology. 2008b; 24(12):2074-2080.
25. Kuddus M, Ramteke PW. Production optimization of an extracellular cold-active alkaline protease from Stenotrophomonas maltophilia MTCC 7528 and its application in detergent industry. African Journal of Microbiology Research. 2011; 5(7):809-816.
26. Kuddus M, Ramteke PW. Recent developments in production and biotechnological applications of cold-active microbial proteases. Critical reviews in microbiology. 2012; 38(4):330-338.
27. Kulakova L, Galkin A, Kurihara T, Yoshimura T, Esaki N. Cold-active serine alkaline protease from the psychrotrophic bacterium Shewanella strain Ac10: gene cloning and enzyme purification and characterization. Applied and environmental microbiology. 1999; 65(2):611-617.
28. Kumar CG, Takagi H. Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnology advances. 1999; 17(7):561-594.
29. Luisa Tutino M, di Prisco G, Marino G, de Pascale D. Cold-adapted esterases and lipases: from fundamentals to application. Protein and peptide letters. 2009; 16(10):1172-1180.
30. Marchi P, Longhi V, Zangrossi S, Gaetani E, Briani F, Dehò G. Autogenous regulation of Escherichia coli polynucleotide phosphorylase during cold acclimation by transcription termination and antitermination. Molecular Genetics and Genomics. 2007; 278(1):75-84.
31. Margesin R. Biotechnological Applications of Cold-Adapted Organisms: With 65 Figures and 45 Tables. Springer Science & Business Media. 1999.
32. Margesin R, Dieplinger H, Hofmann J, Sarg B, Lindner H. A cold-active extracellular metalloprotease from Pedobacter cryoconitis—production and properties. Research in microbiology. 2005; 156(4):499-505.
33. Margesin R, Feller G. Biotechnological applications of psychrophiles. Environmental technology. 2010; 31(8-9):835-844.
34. Margesin R, Feller G, Gerday C, Russell NJ. Cold-adapted microorganisms: adaptation strategies and biotechnological potential. Encyclopedia of environmental microbiology. 2002.
35. Margesin R, Schinner F. Properties of cold-adapted microorganisms and their potential role in biotechnology. Journal of Biotechnology. 1994; 33(1):1-14.
36. Marx J-C, Collins T, D'Amico S, Feller G, Gerday C. Cold-adapted enzymes from marine Antarctic microorganisms. Marine biotechnology. 2007; 9(3):293-304.
37. Moon SH, Parulekar SJ. A parametric study ot protease production in batch and fed-batch cultures of Bacillus firmus. Biotechnology and bioengineering. 1991; 37(5):467-483.
38. Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NP. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiology and molecular biology reviews. 2006; 70(1):222-252.
39. Mozhina N, Burmistrova O, Pupov D, Rudenskaya G, Dunaevsky YE, Demiduk I. Isolation and properties of Serratia proteamaculans 94 cysteine protease. Russian Journal of Bioorganic Chemistry. 2008; 34(3):274-279.
40. Parrilli E, De Vizio D, Cirulli C, Tutino ML. Development of an improved Pseudoalteromonas haloplanktis TAC125 strain for recombinant protein secretion at low temperature. Microbial cell factories. 2008; 7(1):2.
41. Rao MB TA, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev. 1998; 62(3):597-635.
42. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiology and molecular biology reviews. 1998; 62(3):597-635.
43. Reddy G, Matsumoto G, Shivaji S. Sporosarcina macmurdoensis sp. nov., from a cyanobacterial mat sample from a pond in the McMurdo Dry Valleys, Antarctica. International Journal of Systematic and Evolutionary Microbiology. 2003; 53(5):1363-1367.
44. Russell RJ, Gerike U, Danson MJ, Hough DW, Taylor GL. Structural adaptations of the cold-active citrate synthase from an Antarctic bacterium. Structure. 1998; 6(3):351-361.
45. Saba I, Qazi PH, Rather SA, Dar RA, Qadri QA, Ahmad N *et al*. Purification and characterization of a cold active alkaline protease from Stenotrophomonas sp., isolated from Kashmir, India. World Journal of Microbiology and Biotechnology. 2012; 28(3):1071-1079.
46. Siddiqui KS, Cavicchioli R. Cold-adapted enzymes. Annu Rev Biochem. 2006; 75:403-433.
47. Suzuki S, Odagami T. Low-temperature-active thiol protease from marine bacterium Alteromonas haloplanktis. Journal of marine biotechnology. 1997;

5(4):230-233.
48. Taguchi S, Ozaki A, Momose H. Engineering of a cold-adapted protease by sequential random mutagenesis and a screening system. Applied and environmental microbiology. 1998; 64(2):492-495.
49. Van Den Burg B. Extremophiles as a source for novel enzymes. Current opinion in microbiology. 2003; 6(3):213-218.
50. Vázquez S, Hernández E, Mac Cormack W. Extracellular proteases from the Antarctic marine Pseudoalteromonas sp. P96-47 strain. Rev Argent Microbiol. 2008; 40(1):63-71.
51. Yan B-Q, Chen X-L, Hou X-Y, He H, Zhou B-C, Zhang Y-Z. 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. 2009; 13(4):725-733.
52. Zeng R, Zhang R, Zhao J, Lin N. Cold-active serine alkaline protease from the psychrophilic bacterium Pseudomonas strain DY-A: enzyme purification and characterization. Extremophiles. 2003; 7(4):335-337.