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MINI-REVIEW

Cold survival strategies for bacteria, recent advancement and potential industrial applications

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Abstract

Microorganisms have evolved themselves to thrive under various extreme environmental conditions such as extremely high or low temperature, alkalinity, and salinity. These microorganisms adapted several metabolic processes to survive and reproduce efficiently under such extreme environments. As the major proportion of earth is covered with the cold environment and is exploited by human beings, these sites are not pristine anymore. Human interventions are a great reason for disturbing the natural biogeochemical cycles in these regions. The survival strategies of these organisms have shown great potential for helping us to restore these pristine sites and the use of isolated cold-adapted enzymes from these organisms has also revolutionized various industrial products. This review gives you the insight of psychrophilic enzyme adaptations and their industrial applications.

Keywords Psychrophiles · Cold-active enzyme · Industrial applications · Bioremediation · Anti-freezing proteins

Introduction

Through the course of evolution, bacteria have evolved themselves to survive in extreme environmental conditions. Extremophiles, microorganisms surviving in extreme physical and geochemical conditions such as broader temperature range (sub-zero to more than 100 °C), high salinity (up to 5M salt concentration) (Kamekura 1998) and pressure (sometimes up to 50 MPa) (Kato et al. 1998), which are detrimental to most of the life present on earth. A unique group of such extremophilic microorganisms that have evolved to colonize permanently cold habitats including deep oceans (Xu et al. 2003; Yang and Dang 2011; Groudieva et al. 2004), high mountains and polar areas are psychrophiles (Buzzini et al. 2012; De Los Rios et al. 2006). These microorganisms are found in soil (Soares et al. 2012) water or associated with plant and animals (Dalmaso et al. 2015). By definition, the optimal temperature for these microorganisms is considered to be around 15 °C or less and maximum growth temperature around 20 °C, however, they can also show a significant rate of proliferation below or near 0 °C (Morita 1975). Recently, a bacterium, isolated from arctic permafrost named *Planococcus halocryophilus*, was reported to proliferate at temperature − 20 °C and metabolically active at temperature − 25 °C (Mykytczuk et al. 2013). The deleterious effect of the low temperature particularly targets the cell membrane permeability, thus restricting the flexibility of the membrane (Goodchild et al. 2004; Ratkowsky et al.2005). The proliferation capability of psychrophiles at very low temperature indicates that they have adapted certain molecular and morphological changes to survive and multiply optimally under extreme conditions (Ratkowsky et al. 1982; Wiebe et al. 1992; Ayala-del-Río et al. 2010; Aslam et al. 2012; Feng et al. 2013). Though biological activity in an extremely cold area such as polar regions seems to be limited, psychrophiles have successfully expanded themselves in these areas by adapting alterations at various levels including membranes, proteins, and enzymes, empowering them to offset the detrimental effects of low temperature (Anguilar et al. 2001; Barria et al. 2013; Chattopadhyay and Jagannadham 2001). These microorganisms attracted investigators particularly because of their enzymatic ability to work in extreme cold

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conditions and thus provided the enormous natural resources that can be used in vitro for various purposes (Damhus et al. 2013; Mukhopadhyay et al. 2015; Lee et al. 2017). Enzymes from these microorganisms have been particularly used in industries such as food (improvement of milk fermentation and meat and dough quality), agriculture (as biofertilizers), and textile (dye removal or bleaching process) (Fenney and Yeh 1998; Yeh et al. 2009; Sun et al. 1995; Gurung et al. 2013; Nigam 2013).

Enzymatic adaptations

Enzyme kinetics

Arrhenius equation used to define the rate of chemical reaction indicates an inverse relationship between the rate of reaction (K) and activation energy (E_a) and a directly proportional relationship with the absolute temperature (T) (Laidler 1984),

$$K = Ae^{-Ea/RT}. \quad (1)$$

The reaction rate of temperate enzymes falls near to zero when observed at 0 °C (Marshall 1997). The possible reasons contributing to this fall in reaction rate could be changes in pH or lower rate of diffusion of substrate and product (Coquelle et al. 2007; Petrescu et al. 2000). The viscosity of system increases many folds at lower temperature along with the decrease in diffusion rate of both solutes as well as solvents. In general, the enzyme affects the rate of reaction by lowering the activation energy (Siddiqui and Cavicchioli 2006), allowing more substrate to form complexes. In Eq. (1), it is clear that activation energy is quite dependent on the temperature. Low temperature hampers and reduces the diffusion rate for both solvent and solute (Peterson et al. 2007), ensuing a significant increase in required kinetic energy to overcome activation energy barrier. This increase in required kinetic energy eventually slows down the rate of reaction (Marshall 1997). Low temperature surviving organisms have developed the compensatory strategies to bypass the effect of low temperature on their metabolism. These adapted strategies involve an increase in enzyme concentration and evolution of enzymes whose reaction rate would be only diffusion controlled and temperature-independent (Georlette et al. 2004). Study of point mutations disrupting N- terminal non-covalent interactions in *Bacillus* lipase by error-prone PCR method explained the shift in the optimum temperature from (25 °C) towards the low-temperature values (10 °C) (Goomber et al. 2016) and synthesis of enzymes having up to tenfold higher specific activity signifies their maximal activity as well as stability in such extreme cold environments (Feller 2013). Psychrophilic enzymes show trends for optimizing their catalytic efficiency (K_{cat}/K_m) either by increasing K_{cat0} (Wolfenden and Snider 2001; Wolfenden et al. 2011) and decreasing K_m (Coquelle et al. 2007; Fields and Somero 1998) or sometimes improving K_{cat} at the expense of K_m (Feller 2003).

Enzyme stability

Structural stability

Compared to their mesophilic counterparts, psychrophiles have several factors modification in their enzyme structure contributing to lower structural stability (Sindhu et al. 2017; Lee et al. 2016; Yang and Dang 2011; Berlemont et al. 2009). Attenuation of various interaction including ion pairs, H-bonding, ion bonding, charge–dipole interaction, aromatic interaction, and hydrophobic interaction are majorly responsible for the low structural stability (Feller 2003; De Maayer et al. 2014). The presence of arginine residues in mesophiles and thermophiles contributes to the formation of a large number of salt bridges. The comparative account shows the lack of salt bridges in psychrophiles, probably the one factor explaining the trend is the lack of arginine residues (Ramli et al. 2013). Although H-bonds are relatively weak interactions, large number of these bonds results in stability of three dimensional structure of a protein (Creighton 1991). Involvement of the aromatic interaction in mesophilic enzymes (subtilisins or β-lactamase) contributes to their higher stability as compared to their psychrophilic counterparts those have no such interaction (Feller and Gerdy 1997). Another major factor responsible for the high stability of the mesophilic enzyme is due to the bunching of hydrophobic side chains within the core of the protein. Substitution within the hydrophobic core of proteins has shown the trend of decreasing hydrophobicity index in psychrophiles (Feller et al. 1994; DasSarma et al. 2013).

Thermal stability

Reduced thermal stability has been observed almost in all cold-adapted enzymes (Feller 2013; Maiangwa et al. 2015). The major heat-labile elements are active sites present in these enzymes (Georlette et al. 2004; Collins et al. 2003; Fields and Somero 1998). Many of membrane-bound or unbound (Marshall 1997) enzymes have shown the huge temperature sensitivity possibly because of the distortion in the lipid bilayer and its associated proteins (Arcus et al. 2016). Cooperative unfolding at a higher temperature for cold-adapted proteins has also been observed for low molecular weight proteins (Siddiqui and Cavicchioli 2006). In large enzymes, such as α-amylases from *P. haloplanktis* showed that cooperative unfolding appears due to a small number of interactions responsible for the structural integrity (D'Amico et al. 2001). Another

important phenomenon that occurs usually at a temperature below T_{max} disturbs the enzyme structure and interferes with reaction rate at a lower temperature is cold denaturation of enzymes (Siddiqui and Cavicchioli 2006; Aznauryan et al. 2013; Gulevsky and Relina 2013). These are the temporary or reversible changes in the structure of a protein that is responsible for the loss of enzymatic activity at a lower temperature. Cold denaturation is the result of the disruption of hydrophobic weak interactions that are responsible for the protein folding at a lower temperature (Graziano 2014; Marshall 1997). Another outcome observed in cold denaturation involves the hydration of non-polar and polar groups (Vajpai et al. 2013; Siddiqui and Cavicchioli 2006) which clearly suggests that psychrophilic enzymes or cold-adapted enzymes must be resistant to cold denaturation. It also suggests that hydrophobic interactions are of less importance in cold-active enzymes (Marshall 1997).

Enzyme flexibility

The structural and conformational features that provide flexibility to the enzymes in psychrophilic bacteria are generally antagonistic to the more stable and stiff thermophilic and mesophilic counterpart (Siddiqui and Cavicchioli 2006; Violotet al. 2005). The various adaptations acquired by psychrophilic enzymes include a decrease in the enzyme'score hydrophobicity while increase in the same at the surface as compared to the thermostable enzyme (Badieyan et al. 2012). The presence of weaker inter-subunit interactions and inter-domain decreased secondary structures, longer loops along with fewer electrostatic interactions and less number of disulfide bridges, all together confer the high flexibility to the psychrophilic enzymes (Marx et al. 2007; Cavicchioli et al. 2011). The flexibility in such proteins can be explained as global and local flexibility (Fields and Somero 1998). Global flexibility is overall conformational flexibility while local flexibility is confined to a distinct part of a particular protein. It has also been postulated that global flexibility might be responsible for the increase in the activity and low stability at the risk of increasing incorrect folding sometimes (D'Amico et al. 2001). On the other hand, local flexibility has been explained by the studies showing thermal unfolding of proteins starting from the most flexible part such as extremities, surface or active site on a particular protein (Siddiqui and Cavicchioli 2006).

Enzyme activity

Studies on enzymes from the extreme cold climates have shown the molecular modifications to nullify the detrimental consequences of low temperature on the specific activity of these cold-active enzymes (Ramírez-Sarmiento et al. 2013; Rivkina et al. 2000; Dick et al. 2016). Considering the energetics of cold activity, low temperature can greatly reduce the rate of reaction by increasing the free activation energy (Tattersall et al. 2012). To decrease the amount of this energy hurdle and compensate any detrimental effect, cold-adapted enzymes have shown some survival strategies (Lian et al. 2015; Garsoux et al. 2004; Fedoy et al. 2007), like they tend to increase their K_m to increase the reaction rate (Feller and Gerday 1997). The free activation energy, i.e., $\Delta G^{\#}$ is the obstruction between the ground state and transition state, and lower the energy barrier, greater will be the rate of reaction resulting induced enzymatic activity. Considering the transition state theory when enzyme encounter substrate, ES complex is formed which falls into an energy pit (higher the affinity of enzyme to its substrate, higher the energy required by ES to reach $ES^{\#}$); to proceed the reaction further, ES reach to an activated state $ES^{\#}$ that eventually breaks down into enzyme and product,

$$E + S \rightleftharpoons ES \rightleftharpoons ES^{\#} \rightarrow E + P. \quad (2)$$

In cases of cold-adapted enzymes, to reduce this energy barrier into two compensatory strategies have been studied. In first, it has been observed that affinity for the substrate is weak, resulting in decrease in magnitude of the energy barrier and ultimately enhancing the enzyme activity (Feller 2013). Second, survival strategy corresponds to energetics of cold activity. The classical Gibbs–Helmholtz explains the dependency of free energy of activation on both the enthalpy and entropy,

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#}. \quad (3)$$

Also considering the transition state theory, enzymatic activity, K_{cat} shows a relation to temperature and free energy activation as follows (Siddiqui and Cavicchioli 2006):

$$K_{cat} = (K_B T/h)\mathrm{e}^{-\Delta G^{\#}/RT}. \quad (4)$$

Equations (3) and (4) can together be summarized to consider the effect of $\Delta H^{\#}$ and $\Delta S^{\#}$ directly on the enzymatic activity (K_{cat}) by the following equation (Eyring 1935):

$$K_{cat} = (K_B T/h)e^{-\{(\Delta H^{\#}/RT)+(\Delta S^{\#}/R)\}}. \quad (5)$$

Lower the value of $\Delta H^{\#}$ (Enthalpy) or higher the $\Delta S^{\#}$ (Entropy), lower will be the $\Delta G^{\#}$ and thus lesser will be the rate of reaction reduced at a lower temperature (Lonhienne et al. 2000). This feature of reduced $\Delta H^{\#}$ as compared to their mesophilic counterparts has been observed almost in all psychrophilic enzymes (Feller 2013). This reduction in activation enthalpy is structurally achieved by decreasing the enthalpy related interaction need to be broken at the time of transition state formation that in turn enhance the flexibility in the active site of the enzymes (Siddiqui and Cavicchioli 2006).

Activity–stability–flexibility relationship

Comparing psychrophiles with their thermophilic counterparts which are characterized by high thermostability due to the conspicuous molecular rigidity leading to the weak specific activity, these cold-active enzymes show great complementarities at low energy cost due to highly flexible structure and thus resulting in high specific activities (Gerdy et al. 2000). The activity–stability–flexibility trade-off implies that arise in enzyme activity is assisted by a reduction in enzyme stability. This assumption is based on thermophilic homologous enzymes showing low activity in surrounding temperatures and cold-active enzymes showing high intrinsic activity linked to increased thermo-liability (Siddiqui 2017). Site-directed mutagenesis of the enzyme subtilisin from cold-adapted Antarctic *Bacillus* strain TA39 explained the complexity of the relationship between activity and flexibility by revealing that some elements of enzyme structure control protein stability while other regions confer the flexibility giving optimal catalytic efficiency. The speculations were made by observing increased firmness of molecular assemblies through the addition of aromatic interactions, disulfide and salt bridges and by an increase in affinity of the enzyme for calcium ions by modification of a calcium ligand responsible for protection against thermal denaturation. A cooperative enhancement in specific activity and overall activity was also observed when compared to the mesophilic subtilisin, demonstrating the fact that thermostability is not inversely related to the specific activity (Narinx et al. 1997). In another study, an enzyme from *Pseudomonas mandelii* named esterase EstK demonstrated the flexibility–stability trade-off. Mutation in the conserved residues D308-Y309 positioned in the loop of catalytic H307 residue in the enzyme resulted in increased conformational flexibility. These mutants showed higher catalytic rate and substrate affinity when compared to the wild-type estraseEstk via enlargement of the active site at the expanse of reduced thermal stability (Truongvan et al. 2016). Mutations in proteins that create a balance between the local active site flexibility and overall rigidity are considered to be adaptive as they promote both enzymatic activity and thermal stability (Kokkinidis et al. 2012). Surface exposed, irregularly structured, and flexible protein loops are related to the stability and function in many proteins (Henzler-Wildman and Kern 2007; Goodey and Benkovic 2008) (Fig. 1).

Membrane fluidity

Structural integrity of the membrane is dependent function of the fluidity of the membrane (Deming 2002). The lipid composition of the membrane confers the physical properties of the membrane and low temperature has an adverse effect on it (D'Amico 2006). Low temperature solidifies (gel phase transition) the membrane thus resulting in the loss of functions of membrane. Lower growth temperatures facilitate the production of shorter acyl chain (rather than head group) length, methylated branched fatty acids and higher content of polyunsaturated or simply unsaturated fatty acids (Chintalapati et al. 2004). Such adaptive compositions of the membrane increase the fluidity of membrane by introducing a steric constraint that reduces the number of interactive forces in the membrane. Other adaptations that potentially increase the fluidity of membrane include an increase in the content of huge lipid head groups and proteins (Chintalapati et al. 2004). A two-component signal transduction system was identified in *Bacillus subtilis* involving DesK (a sensor Kinase) and DesR (a response regulator) proteins. Assumptions from the study were made that DesK assume different states in response to temperature-dependent alterations in membrane fluidity by regulating kinase to phosphatase activity ratio. Phosphatase-dominant state is present at temperature 37 °C when membrane lipids are disordered and vice-versa. DesR in response to DesK binds to *des* gene responsible for the transcription of Δ5-lipid desaturase in the bacteria. These newly synthesized unsaturated fatty acids cause disorder in the lipid membrane even at lower temperature and further act as negative regulator for the *des* gene (Anguilar et al. 2001) (Fig. 2).

Comparative bioinformatical analysis of membrane proteins from psychrophiles and mesophiles has revealed the compositional changes of amino acid in psychrophiles. One major finding of the study showed an increase in Isoleucine in the part of the sequences those located outside in the bilayer may be due to hydrophobic and helix-destabilizing characteristic of Isoleucine responsible for the decreased stability and increase flexibility. Also studies showed a trend in decreased amount of alanine in the membrane proteins, resulting in less helix formation and thus decreasing the stability (Kahlke and Thorvaldsen 2012). Transcriptomic analyses in various studies also revealed the up regulation of genes inducing synthesis of peptidoglycans, lipopolysaccharides and other membrane proteins, with a common motive of counteracting the effect of lower temperature on membrane fluidity (Frank et al. 2011; Deming 2002). Other studies suggesting the factors responsible for modulating the membrane fluidity also include wax esters and polar, non-polar carotenoids

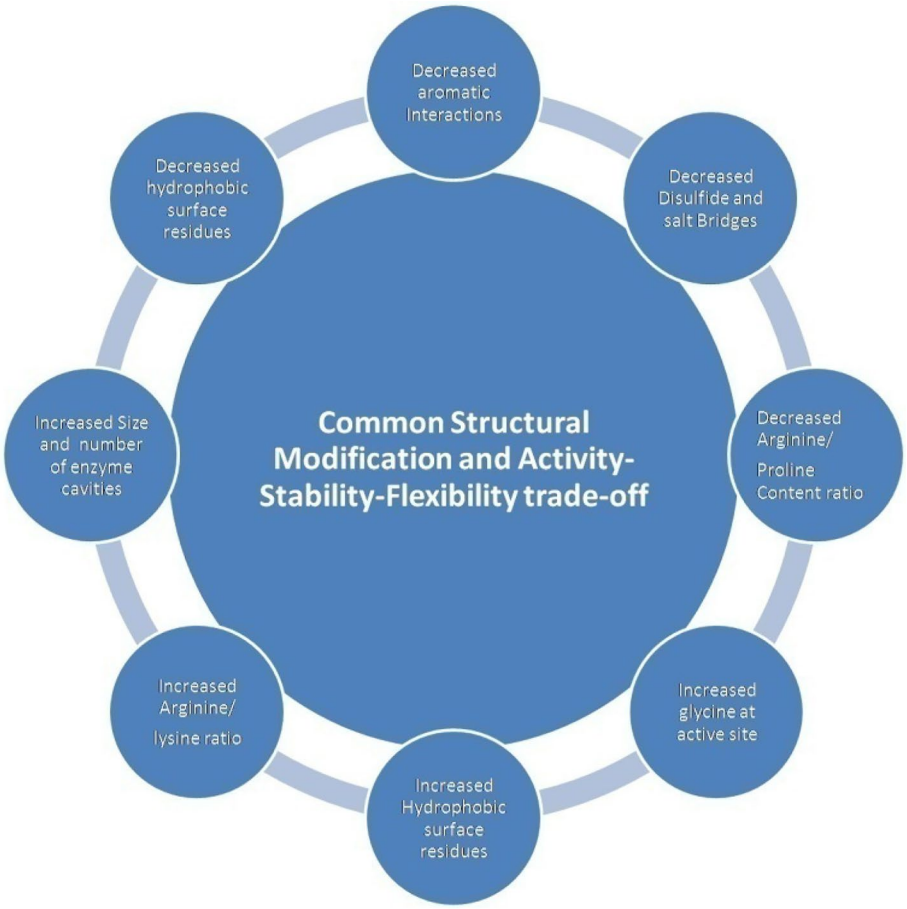

Fig. 1 Common adaptations facilitating survival of bacteria in extreme cold environment. Altered structural and functional modifications in proteins and enzymes (shown in the figure) are major factors responsible for activity–flexibility–stability trade-off in psychrophilic group

present in the membrane (Rodrigues et al. 2008; Chattopadhyay 2006) (Fig. 3).

Cold-adapted enzyme

Ice-binding proteins

Ice-binding proteins such as ice nucleating proteins (INPs) and antifreeze proteins (AFPs) exist in several organisms including bacteria (Raymond et al. 2007), insects (Kristiansen et al. 2011), fungi (Xiao et al. 2010), plant (Middleton et al. 2009), and yeast (Lee et al. 2010). AFPs particularly bind to tiny ice crystals to prevent further recrystallization and growth of ice that could be life threatening otherwise (D'Amico 2006). These polypeptide chains get adsorb to the expanding ice front and thus restricting its growth to the regions between adsorbed polypeptide chains (Raymond and DeVeries 1977). AFPs binding to ice crystals do not actually retard the growth of ice front instead they limit the growth to a manageable size for a longer period between the melting point and freezing point. The difference between the two is called thermal hysteresis, which inhibits the thermodynamically favorable growth of ice crystals (D'Amico 2006). Unlike AFPs, the INPs work by inducing the formation of tiny ice crystals and prevention of larger ice crystals (Sally et al. 2010; Lorv et al. 2014). Ice nucleating proteins in bacteria are encoded by structural gene-expressing membrane-bound proteins. These INPs help in the formation of ice at high subzero temperatures (− 2 to − 10 °C) (Lee et al. 1995). Dumen and Olsen (1993), discovered first ever AFPs from *Micrococcus cryophilus* and *Rhodococcuserythropolis*. Since then a lot of research has been done for the isolation, characterization and identification of various ice-binding proteins. In 1995 and 1998, two glycoproteins from *Pseudomonas putida* GR12-2 of molecular weight ~ 34 kDa and 164 kDa, respectively were characterized (Sun et al. 1995; Xu et al. 1998). AFP isolated from Antarctic bacteria *Colwellia* sp. strain SLW05 (*Col*AFP) showed thermal hysteresis activity at 4 °C at a concentration of 0.14 mM salt concentration (Hanada et al. 2014). Usually an ice-binding protein is small, single domain and soluble but in a recent study, characterization of a IBP from Antarctic bacterium *Marinomonas primoryensis* (*mp*IBP) was found exceptionally long (molecular weight of 1.5MDa) comprising of five different domains, out of which only fourth domain was responsible for the antifreeze activity, suggesting that this protein might also be

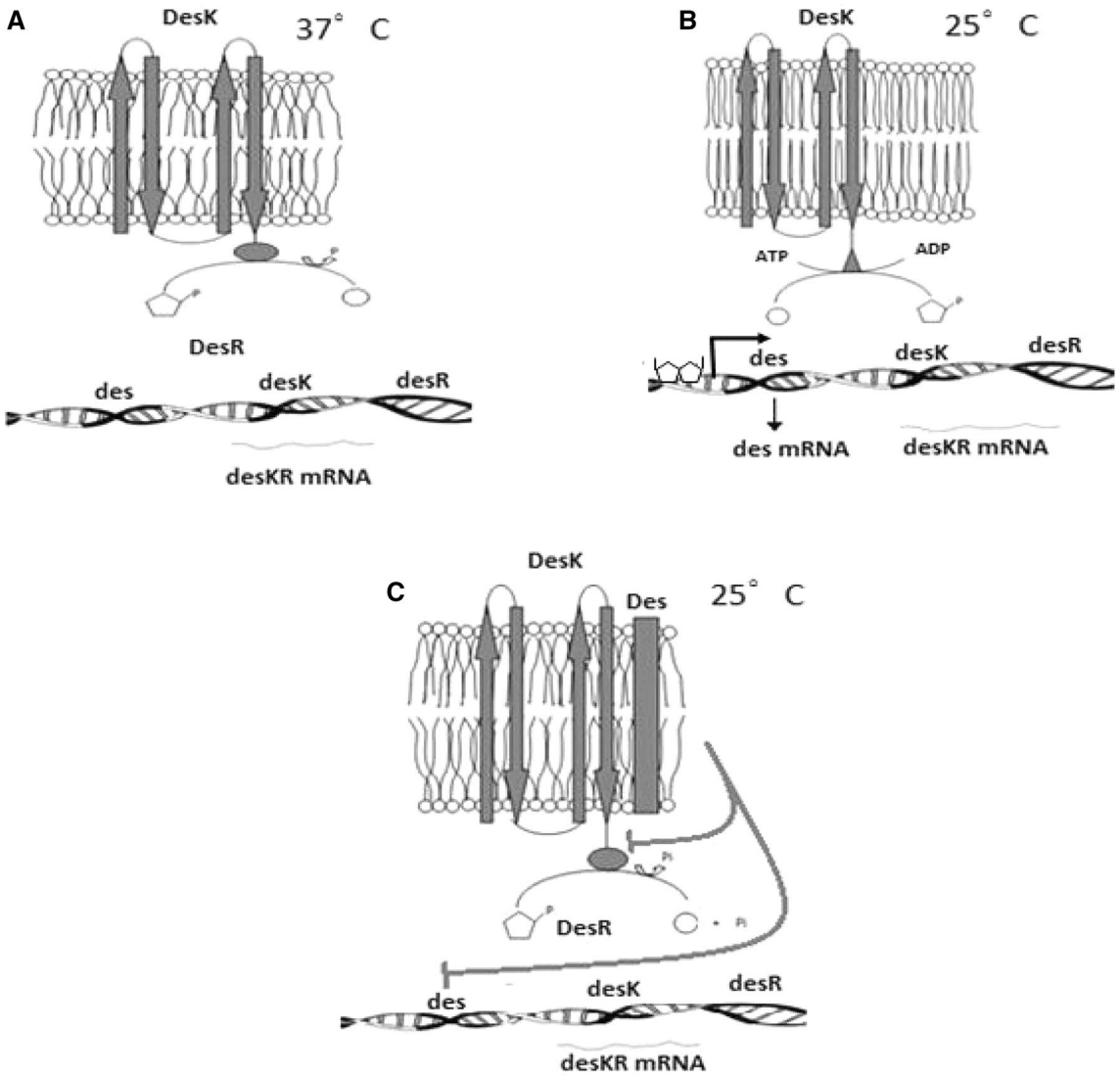

Fig. 2 Two-component signal transduction system in *Bacillus subtilis*. **a** Membrane lipids are present in disordered state at temp 37 °C because of phosphate dominated state of DesK. **b** DesR binds to *des* gene and starts the transcription of *des* (Δ5-lipid desaturase) at temperature 25 °C or below. **c** Des cause the disorder of the lipid membrane at low temperature and simultaneously act as negative regulator of *des* gene

involved in some distinct functioning for the bacteria (Dolev et al. 2016). Other AFPs from *Marinomonasprimoryensis* (*mp*AFP) showed evidences of cooperativity and producing over 2 °C freezing point depression. This protein was unique because it does not crystal faceting during thermal hysteresis (Gilbert et al. 2005) (Fig. 4).

Alpha amylase

α-Amylases are always proven to be excellent model for the study of cold adaptations in the enzymes (Vester et al. 2015; Aghajarietal. 1998; Mahdavi et al. 2010; Cipolla et al. 2011). These enzymes play an important role in industrial application and provide 25% of the total enzyme market (Sindhu et al. 2017). A thorough comparative structural study of the α-amylase from *A. haloplanktis* (AHA) with the human α-amylase has provided insight of the cold adaptations at molecular level. Loop region variations in this particular enzyme explained the cold adaptations supporting activity–stability–flexibility trade-off and factors responsible for enzyme specificity (Aghajari et al. 1998). Biochemical characterization and molecular cloning of α-amylase (Amy-E) isolated from cold-adapted *Exiguobacterium* sp. SH3 showed novel halotolerant psychrophilic features. Activity of Amy-E was stimulated to 103% at salt concentration of upto 5M NaCl (due to non-ionic surfactant) and retained the 41% of the activity at temperature 0 °C. In addition, the enzyme was found stable against denaturants such as SDS, acetone,

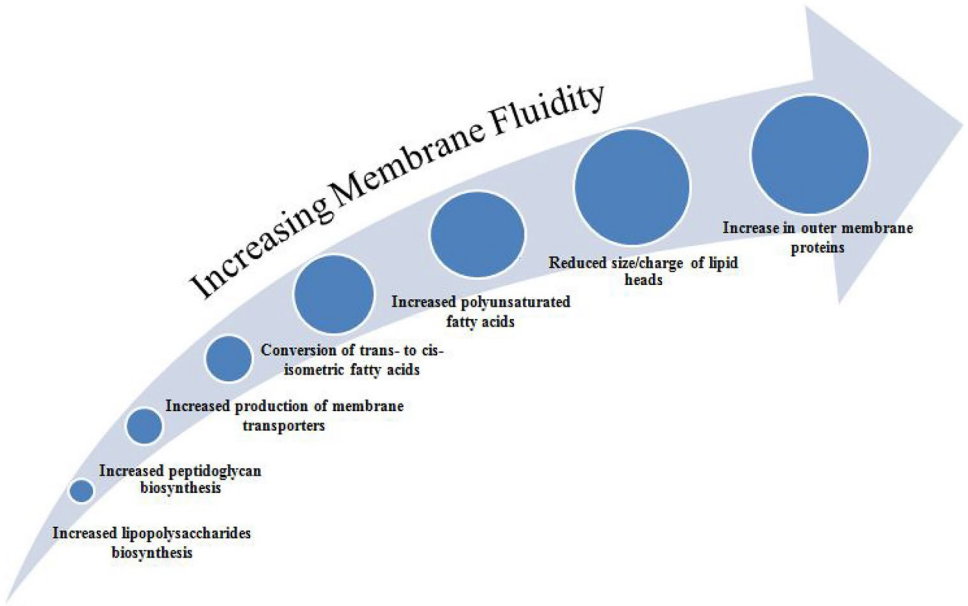

Fig. 3 Common structural adaptations accounting for membrane fluidity in psychrophiles. The arrow indicates increase in the membrane fluidity in psychrophilic group with the addition of each membrane modification

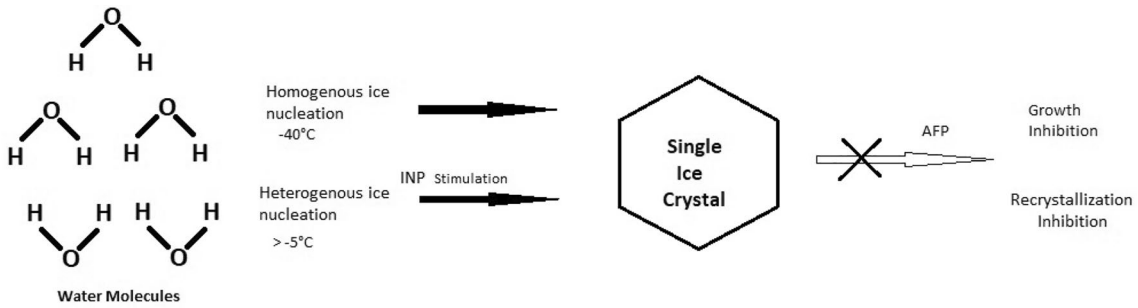

Fig. 4 Schematic representation of mode of action of ice nucleating and anti-freezing proteins. INPs are responsible for homogenous and heterogeneous nucleation of ice at temperature − 40 °C and > − 5 °C forming tiny ice crystals. AFP inhibits the further ice growth and limits the size of ice crystals formed

EDTA and alcohol (Emampour et al. 2015). In another study, a novel α-amylase (BiLA) with optimum activity at 20 °C and pH 5 was cloned and characterized from the psychrophilic bacteria *Bifidobacterium longum*. Kinetic analysis observed highest catalytic efficiency for amylose as a substrate. Breakthrough in their study revealed that the enzyme was capable of producing slow digestible starch when provided with starch as a substrate, indicating a potential application in food industries (Lee et al. 2016). A multiple mutational study of mutants generated from α-amylase (AHA) of psychrophilic Antarctic bacterium *Pseudoalteromonas haloplanktis*, successfully unveiled the factors responsible for kinetically and thermodynamically driven stability, kinetics of protein folding and thermal denaturation (Cipolla et al. 2011). Cold-adapted α-amylase isolated from Antarctic *Arthrobacter agilis* was cloned and expressed heterogeneously showed optimal activity at 30 °C and retained high activity at broad temperature (30–60 °C) range unlike other cold-adapted enzymes (Kim et al. 2017). In a separate study conducted in 2016, modular domain engineering (truncation of N-terminal domain) of alkaline α-amylase (Amy703) from *Bacillus pseudofirmus*703 showed improved specific activity and thermo-stability with significant improvement in the K_{cat} and K_{cat}/K_m (Lu et al. 2016) (Fig. 5).

Esterase

From food industries to environmental biotechnology and medical industries, estrases are considered as important enzyme for their role in related fields (Joseph et al. 2008; Jeon et al. 2009; Fan et al. 2017). Various attempts have been made and successfully proven estarses as an excellent model enzyme for the study of cold adaptations at molecular level (Jiang et al. 2016; De Santi et al. 2016; Hong et al. 2012; Novototskaya-Vlasova et al. 2012). The role of aromatic (Trp and Tyr) residues present in the active site of esterase

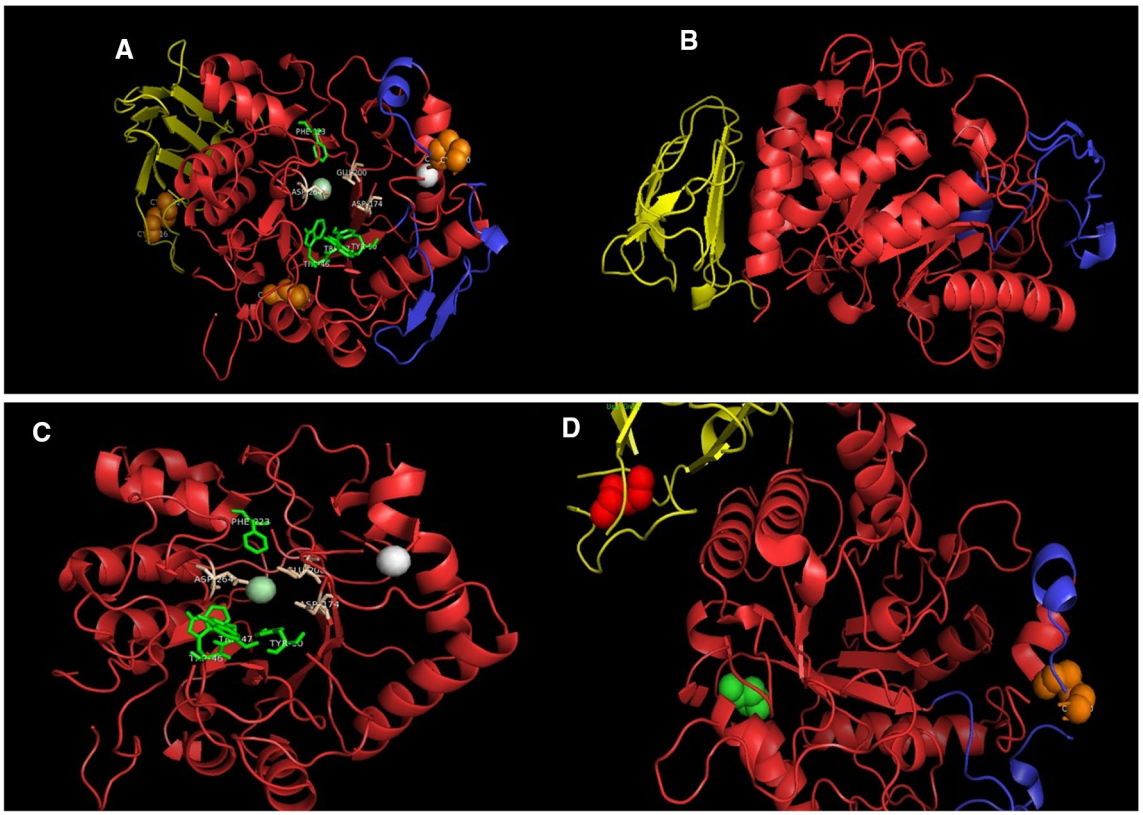

Fig. 5 **a** Complete structure of psychrophilic α-amylase (*A. halo-planktis*) (PDB ID: 1AQH), **b** Three domains of α-amylase; domain A (middle red region, 1–86 and 130–356), domain B (right blue region, 87–129), domain C (left yellow region, 357–448), **c** active site residues of α-amylase; catalytic triad (Tint wheat color-Asp264, Glu200, Asp174) and aromatic residues (green color) conserved Tyr50, Trp46 and Trp47 with chloride ion (pale green sphere) and calcium ion (white color residue), **d** disulfide bridges (spheres); Cys20–Cys74 (green), Cys120–Cys137 (orange), Cys402–Cys416 (red)

EstSP1 from *Sphingomonas glacialis* was investigated. Point mutation of Tyr191 to Trp, His, Ala and Phe showed reduced conformational flexibility and catalytic activity of enzyme at lower temperature at the expense of increasing stability, indicating its role in cold adaptation for the enzyme EstSP1 (Kashif et al. 2017). Comparative study by molecular dynamics of four esterases (Est2 from thermophilic bacterium *(A) acidocaldarius*, EstB from mesophilic *(B) thailandensis*, EstP from psychrotrophic *Pseudomonas* spB11-1and EstS from psychrophilic *S. halifaxensis*) isolated from bacteria surviving in temperature range from 10 to 70 °C showed effect of different temperatures on the activity and substrate specificity (p-nitrophenyl esters of fatty acids) of these enzymes. EstS activity was found optimum at temperature near 25 °C and decreased further as the temperature increased, indicating its psychrophilic nature (Kovacic et al. 2015). Characterization and cloning of cold-adapted esterase (EstPc) from psychrotrophic bacteria *Psychrobacter cryohalolentis K5*^{T} revealed its maximum activity (substrate—p-nitrophenyl butyrate) at temperature 35 °C and pH 8.5, however, assays at different temperatures also revealed the retaining of 90% of its maximum activity at temperature between 0 and 5 °C (Novototskaya-Vlasova et al. 2012). A novel cold-adapted esterase (ThaEst2349) from marine psychrophilic bacterium *Thalassospira* sp. GB04J01 was characterized. Crystal structure resolved to 1.69 Å revealed a biological unit with two peptide chains and a characteristic cap domain consisting of catalytic triad (Ser158, His285 and Asp255). Structural analysis of enzyme compared to thermophillic counterparts (*Pyrobaculum calidifontis* VA1, *Sulfolobus tokodaii and Alicyclobacillus acidocaldarius*) explained the cold-adapted nature of the novel enzyme by revealing the presence of higher content of methionine and lower number of hydrogen bond and ion pairs responsible for higher flexibility at lower temperature (De Santi et al. 2016).

Lipase

Microbial cold-active lipases are one class of enzymes with inherent activity–flexibility–stability property that captures the attention of investigators in past decades for a better understanding of molecular adaptations in cold-adapted microorganisms (Maiangwa et al. 2015). Several cold-active lipases have been identified and characterized till date to

understand the molecular adaptations in the enzyme and their potential application in different industries (Joseph et al. 2011; Li et al. 2013; Leonov 2010; Wi et al. 2014; Ji et al. 2015; Do et al. 2013). Lipopolysaccharide interaction with the lipid hydrolases in cold environment produces a major challenge in purification of cold-active lipases from inhabiting bacteria (Gerday et al. 2000). Several optimization strategies have been developed to ease the challenge of these bacteria to obtain cold-active lipase in purified forms (Basheer and Thenmozhi 2010; Nagarajan 2012; Iftikhar et al. 2011; Wang et al. 2012). Recombinant DNA methods, cloning, X-ray crystallography and bioinformatical analyses have been proven successful for understanding the molecular/structural adaptations in these proteins (Novototskaya-Vlasova et al. 2012; Do et al. 2013; Ali et al. 2013; Maraite et al. 2013). Two different lipases (*Lip-948, Lip-1452*) encoded by lipolytic gene identified in *Psychrobactor* sp. were cloned and expressed with detailing of their primary structure (Xuezheng et al. 2010). Multiparameter study of an extracellular cold-active lipase from *Pseudoalteromonas sp*. NJ 70 isolated from Antarctic sea ice showed specific feature of 31% activity retention under 0 °C and no effect of oxidant H_2O_2 on enzyme activity (Wang et al. 2012). Structural adaptations of cold-active lipase (LipAMS8) from psychrophilic *Pseudomonas* sp., were revealed by predicting the structure of enzyme using bioinformatics tools. Results explained that the N terminus catalytic domain was more responsible for its stability at temperature 0–5 °C than the noncatalytic C terminus (Ali et al. 2013). Ganjalikhany et al. (2012) made an attempt to look into the mechanism of action of cold-active lipase B from *Candida Antarctica* at the molecular level. The results obtained described the alterations in flexibility of enzyme's lid (α5, residues 141–147) region, suggesting functional motions (open–closed conformation) were required to the enzyme's lipolytic activity (Table 1).

Applications

Psychrophilic enzymes being capable of working at very low temperature have revolutionized the industrial area and increased their commercial requirements (Bialkowska et al. 2009; Mukhopadhyay et al. 2015; Ramnath et al. 2016). The evolved adaptations of psychrophilic bacteria have further been exploited and improved for the purpose of industrial and biotechnological applications (Cavicchioli et al. 2011). Various unusual properties of cold-adapted enzymes such as high stability and activity have been observed in many deep oceanic bacterial strains (Kato et al. 2008; Saito and Nakayama 2004). Genetic as well as chemical modifications offer modified properties of enzymes that intensify their performance and reinforce enzyme properties (Li et al. 2015; Esteban-Torres et al. 2014; Zhang et al. 2016). The directed evolution of cold-active enzyme lipase B from *Candida antarctica* has been used to improve both, the half-life of enzyme inactivation ($t_{1/2}$) as well as its specific activity (K_{cat}) (Zhang et al. 2003). The cold-adapted enzymes have shown great influence on industries such as food, detergent, biotechnological companies, and textiles. Out of several applications in different industries, few are described in the next section (Table 2).

Detergent industry

Cold-adapted enzymes with their ability to hydrolyze substrates (Zheng et al. 2011; Roohi et al. 2013; Kuddus and Ramteke 2012) have proven to be very useful for various purposes in industries such as laundry and dishwasher (Aehle 2007), waste water treatment (TePoele and Van der Graaf 2005), and food and dairy products. Detergent manufacturer *Proctor and Gamble in 2009* and *Laugesen in 2010*

Table 1 Selected psychrophilic enzyme from bacteria with their isolation sources

Enzymes	Bacterium	Isolation source	References
α-Amylase	*Exiguobacterium* sp. SH3	Arctic polar region	Emampour et al. (2015)
	Arthrobacter agilis PAMC 27388	Antarctica King George Island	Kim et al. (2017)
	Pseudoalteromonas arctica GS230	Gaogong Island, China	Lu et al. (2010)
	Pseudoalteromonas haloplanktis	Antarctica seawater	Cipolla et al. (2011)
Esterase	*Thalassospira* sp. GB04J01	Sea floor in Vestfjorden area (Northern Norway)	De Santi et al. (2016)
	Peudomonas mandelii JR-1	Natural mineral water (Gyeongsan, Korea)	Hong et al. (2012)
	Psychrobacter cryohalolentis K5T	Siberian cryopeg	Novototskaya-Vlasova et al. (2012)
Lipase	*Colwellia psychrerythraea 34H*	Arctic marine sea	Do et al. (2013)
	Halomonas sp. BRI 8	Antarctic sea	Jadhav et al. (2013)
	Micrococcus roseus	Glacial soil	Joseph et al. (2011)
	Psychrobacter sp. G	Antarctica sea	Xuezheng et al. (2010)
	Bacillus pumilus	Chukchi Sea (Arctic Ocean)	Wi et al. (2014)

Table 2 Selected psychrophilic enzymes and their industrial application

Enzymes	Bacterium	Industries	References
α-Amylase	*Pseudoalteromonas haloplanktis*	Food industry	Aghajari et al. (1998), Gerdy et al. (2000), and Collins et al. (2005)
Cellulase	*Pseudoalteromonas haloplanktis*	Textile industry	Violot et al. (2005) and Ueda et al. (2010)
β-Lactamase	*Pseudomonas fluorescens*	Pharmaceutical (antibody degradation)	Michaux et al. (2008)
β-Galactosidase	*Anthrobactor sp*	Food and biofuel industry	Bialkowska et al. (2009) and Hildebrandt et al. (2009)
Lipase	*Photobacterium lipolytica*	Detergent, biofuel and pharmaceutical	Jung et al. (2008) and Joseph et al. (2008)
Xylanase	*Pseudoalteromonas haloplanktis*	Textile and paper industry	Van Petegem et al. (2003) and Collins et al. (2005)
Esterase	*Lactobacillus plantarum*	Food industry, bioremediation	Esteban-Torres et al. (2014) and Fan et al. (2017)
Pectinase	*Pseudoalteromonas* sp.	Food industry	Tuyen et al. (2001)

showed a relationship between improved energy conservation and reduced wash temperature. Reduction of wash temperature by just 10 °C (40–30 °C) reported producing 30% reduction in used electricity, i.e., equivalent to 100 g of CO_2 per wash (Nielsen 2005; Nielsen and Skagerlind 2007). Enzymes such as cellulases, amylases, lipases and proteases have been used as additives in detergent for low-temperature washing (Aehle 2007). Enzymes from psychrophilic bacteria such as amylase from glacial water (Sharma et al. 2010) have the potential to enhance the enzyme-based effectiveness of low-temperature cleaning formulations. A particular cold-active enzyme isolated from psychrotolerant *Stenotrophomonas maltophilia* alkaline protease showed improved capability with a commercial detergent and found effective for the removal of various proteinaceous stains at low temperature, however, maximum stability and activity exhibited at 20 °C and pH 10 (Kuddus and Ramteke 2012).

Food industry

Cold-adapted enzymes have numerous applications in food industries in recent times (Ueda et al. 2010; Białkowska et al. 2009). These enzymes can have a greater effect in reducing the unwelcomed reactions that are usually feasible at comparatively higher temperature ranges (Cavicchioli et al. 2011). Heating can instantly inactivate these enzymes and thus these enzymes can be used to perform reactions that include high temperature sensitive substrates, for example, a cold-active collagenase can help in beef tenderization at low temperature between 4 and 25 °C and then inactivated at temperature around 40 °C due to autolysis (Zhao et al. 2012). These properties influence the food industry as it is essential to prevent food spoilage or alteration in the original product's nutritional value that could be heat sensitive. Some notable use of these enzymes can be seen as in milk industry where β-Galactosidase is used to diminish the lactose that would otherwise induce lactose tolerances (Karsova et al. 2002; Mateo et al. 2004). Cold-active enzyme pectinases help fruit juice industry in extraction processes by reducing the viscosity and help to clean the final resultant product (Gerdy et al. 2000; Truong et al. 2016). Proteases are used to tenderize the meat in meat industries and enzymes such as xylanases, proteases, amylase, and cellulases reduce the time of dough fermentation during baking processes and improve aromas and moisture level (Wang et al. 2015; Kim et al. 2011; Lee et al. 2016).

Textile industry

In textile industries cold-active enzymes have shown various applications such as denim finishing and sizing, bleaching, bleach termination, excess dye removal (Gurung et al. 2013; Nigam 2013) and stone washing (Ueda et al. 2010). Degumming of the silk to remove sericin (a proteinaceous substance that covers the raw silk) is generally done in an alkaline solution which is considered to be a harsh treatment. An alternative to this harsh alkaline treatment is the use of cold-active proteases as it can remove the sericin without attacking the fiber (Kuddus and Ramteke 2012). Cellulase pretreatment under appropriate conditions reduces the pill formation as well as increase the durability and softness of the garment. The treatment using cold-active cellulases requires low temperature and less enzyme concentration (Gerdy et al. 2000).

Environmental applications: bioremediation

The high catalytic efficiency and unusual specificity at low temperature make these organisms suitable for bioremediation process. Due to enormous seasonal variations in temperate areas, the potential of these bacteria got reduced in degrading hydrocarbon pollutant. However, the inoculation of these environments, particularly with cold-active enzymes in consortia, helped in boosting the degradation of

these hydrocarbons in various seasons (Gerday et al. 2000). Recently, Cytochrome P450 alkane hydrolases and P450 dependent flevodoxin reductase identified from psychrophilic genome showed capability to hydolyse n-Alkane and degradation of nitrotriazine derivative, respectively, at 5 °C, showing their bioremediation potential at low temperature (Bowman and Deming 2014; Jackson et al. 2007). Earth's cold areas were supposed to be pristine until POP contamination were reported in the 1970s (Risebrough et al. 1976). These environments found to be polluted from many sources including industries, tourist activities or migrating species but the major source of contamination in these regions is considered to be atmospheric transportation (Goutte et al. 2013; Bargagli 2008; Gai et al. 2014; Simonich and Hites 1995). Low-temperature biodegradation of POP's such as pesticides, polychlorinated biphenyls (PCBs), chlorobenzoates (CBAs), chlorobenzenes (CBs) and furans and dioxins have been studied and pathways for the degradation have been elucidated (Bajaj and Singh 2015). Three *Sphingobium* strains, namely *Sphingomonas indicum* B90A, *S. japonicum* UT26 and *S. francens*, have been reported for the degradation of γ-HCH at 4 °C though less efficient than at 30 °C (Zheng et al. 2011). Arctic bacterial isolate *Pseudomonas* Cam-1 showed the biodegradable ability to remove industrial POP's like PCBs with higher rates at 7 °C (Master and Mohn 1998). Biodegradation of 3-Chlorobenzoate at 10 °C (Yun et al. 2007) and biodegradation of di, tri, and tetrachlorobenzene at 5 °C (Rapp and Gabriel-Jurgens 2003) by cold-adapted *Rhodococcus erythropolis* S-7 and *R. erythropolis* MS11, respectively, have been reported. The potential of the cold-adapted degraders has been reported for the biodegradation of oil in alpine habitats. Results of the oil-contaminated sample degradation experiment showed up to 40–60% degradation at 10 °C and up to 80% at 4 °C for various concentrations (500 mg, 1200 mg and 5000 mg) after 8 days (Margesin 2000).

Conclusion

Approximately 80% of Earth is covered by cold habitat which has allowed many organisms to evolve and adapt in such conditions. Enormous diversity of cold-adapted microorganisms and evolutionary adaptational features of their enzymes offer potential economic advantages. Due to their unique adaptations such as enzyme flexibility, membrane fluidity, the presence of anti-freezing proteins, low thermal stability and higher specific activity, these organisms have always fascinated the researchers to investigate for various industrial purposes. A substantial gain in energy conservation in detergent industries to an effort for sustainable restoration of cold habitats, are major achievements of these microorganisms that could play important role for the betterment of earth's climate changes. These bacteria have adapted many specific metabolic pathways for the uptake and metabolisms of hydrocarbon pollutants thus their enzymes have extensively been used for the degradation of hydrocarbons helping in the removal of pollutants such as oil spills and pesticide from the environment.

The biochemical, physiological, and ecological characteristics of these microbes are still in initial phase of scientific exploration and more contribution by these organisms is awaited in future in the field of industrial biotechnology.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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