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REVIEW

Adaptational properties and applications of cold-active lipases from psychrophilic bacteria

Jonathan Maiangwa · Mohd Shukuri Mohamad Ali · Abu Bakar Salleh · Raja Noor Zaliha Raja Abd Rahman · Fairolniza Mohd Shariff · Thean Chor Leow

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Abstract Psychrophilic microorganisms are cold-adapted with distinct properties from other thermal classes thriving in cold conditions in large areas of the earth's cold environment. Maintenance of functional membranes, evolving cold-adapted enzymes and synthesizing a range of structural features are basic adaptive strategies of psychrophiles. Among the cold-evolved enzymes are the cold-active lipases, a group of microbial lipases with inherent stability–activity–flexibility property that have engaged the interest of researchers over the years. Current knowledge regarding these cold-evolved enzymes in psychrophilic bacteria proves a display of high catalytic efficiency with low thermal stability, which is a differentiating feature with that of their mesophilic and thermophilic counterparts. Improvement strategies of their adaptive structural features have significantly benefited the enzyme industry. Based on their homogeneity and purity, molecular characterizations of these enzymes have been successful and their properties make them unique biocatalysts for various industrial and biotechnological applications. Although, strong association of lipopolysaccharides from Antarctic microorganisms with lipid hydrolases pose a challenge in their purification, heterologous expression of the cold-adapted lipases with affinity tags simplifies purification with higher yield. The review discusses these cold-evolved lipases from bacteria and their peculiar properties, in addition to their potential biotechnological and industrial applications.

Keywords Psychrophilic bacteria · Extreme adaptation · Cold-active lipase and applications

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J. Maiangwa · T. C. Leow (✉)
Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Enzyme and Microbial Technology Research Center, Universiti Putra Malaysia Serdang, 43400 Selangor, Malaysia
e-mail: adamleow@upm.edu.my

M. S. M. Ali · A. B. Salleh
Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Enzyme and Microbial Technology Research Center, Universiti Putra Malaysia Serdang, 43400 Selangor, Malaysia

R. N. Z. R. A. Rahman · F. M. Shariff
Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Enzyme and Microbial Technology Research Center, Universiti Putra Malaysia Serdang, 43400 Selangor, Malaysia

Introduction

Psychrophilic microorganisms are cold-adapted with distinct properties from other thermal classes (e.g., thermophiles) (Cavicchioli et al. 2002). Their ubiquity in nature relates to their possession of dynamic cellular processes that ensure their survival, growth and adaptation even to extreme forms of life. Polar environments indeed still represents a dynamic reservoir for habitable psychrophiles normally found in nature colonizing such substantial portion of such extreme terrestrial and aquatic environments as—deep sea/ocean, Antarctic regions, glacial habitats, refrigerated appliances, and on/in plants and animals inhabiting cold regions (Russell 1998; Margesin et al. 2007; Buzzini et al. 2012). More than 90 % of the ocean environments sustain a broad diversity of microbial life and drive the functional capacity of the psychrophilic life. Bio-prospecting of most polar ecosystems was selected for equally diverse indigenous psychrophilic microbial assemblages comprising not only prokaryotes, but also eukaryotes,

plants, animals, archaea, eucarya, protists, bacteria, yeasts, unicellular algae and fungi, viruses, flatworms and flagellates (Cavicchioli 2006; Morgan-kiss et al. 2006; Margesin et al. 2007; Parra et al. 2008; Buzzini et al. 2012; Feller 2013) contributing towards carbon and nutrient cycling, bioremediation, production of secondary metabolites, nutrient turnover, biomass production, and litter decomposition in cold ecosystems (Cummings and Black 1999; Trotsenko and Khmelenina 2005; Methé et al. 2005).

With all their cellular processes mediated in the cold environment, it is imperative that components of the cell including metabolism and protein synthesis are suitably adapted against the impact of the cold-shock environment. The most critical metabolic requirement for withstanding the low temperature's harmful effect is the maintenance of functional membranes, evolving cold-adapted enzymes and synthesizing a range of structural features which endow a high level of flexibility in protein structure enabling biocatalysis, high catalytic efficiency at low temperatures, high degrees of thermolability, lower energy of activation and increased structural flexibility for better substrate access (Thomas and Dieckmann 2002; Siddiqui and Cavicchioli 2006; Siddiqui et al. 2013).

The commercialization of the inherent cellular components of cold-adapted psychrophilic microbes in particular enzymes obtained from them has received great attention. Psychrophilic bacteria have adapted and colonized a variety of cold environments producing cold-adaptive enzymes targeted for their biotechnological potential in detergent and food industries, biotransformation, environmental bioremediation, etc. (Russell 1998) compared to the mesophilic and thermophilic enzymes wherein high activity and interaction between substrates and enzyme are impaired by their high thermostable rigidity (Gerday et al. 2000).

The strategy of adaptation to cold environment is peculiar to microorganisms and their constitutive proteins as well as enzymes. Increased flexibility of molecular structure and high specific activity of cold-adapted enzymes apparently represent their thermosensitivity and a complementary interaction at a reduced energy cost. These and other factors add up to make psychrophilic microorganisms to be considered novel bio-resource for cold-active enzymes and other biological products with a spin-off for biotechnological and catalytic gains (Cavicchioli et al. 2011; Ewert and Deming 2013; Bowman 2013; Siddiqui et al. 2013). Many cold-active enzymes obtained from cold-adapted microorganisms include: protease, lipases, amylases and cellulases which have found wide applications (Aghajari et al. 1996; Alquati et al. 2002; Cieśliński et al. 2005).

Lipases hold huge potential in certain areas of application given their diversity and properties. This review examines cold-adapted lipases from psychrophilic bacteria, their adaptative features, purification strategies and exploitation of their industrial and biotechnological enterprise (Tables 1, 2).

Psychrophilic bacteria

The cold environment is being dominated by an array of aerobic and anaerobic bacteria in great diversity amongst other extremophiles overcoming the adverse effect of the reduced temperature, by developing some molecular shields making them catalytically effective, enabling their survival and maintaining their structural and functional adaptation to such extreme environmental conditions with desired properties (Karner et al. 2001; Deming 2002; Ramteke et al. 2005). Psychrophilic bacteria perform basic functions at frozen environments far below 0 °C and others tolerating such latitude with growth rates at 2–12 °C, with some bacteria becoming more piezophilic at 10 °C (Xu et al. 2003).

Diverse groups of bacteria belonging to the Gram-negative α-, β- and γ-proteobacteria from the forest soil, arctic alpine-tundra soil, stream water, mire sediments, lichen, snow algae and Antarctic lakes have been reported to survive cold-active environments (Gilbert et al. 2004; Männistö and Häggblom 2006). Others include those belonging to the *Burkholderia* sp., *Collimonas* sp., *Pedobacter* sp., *Janthinobacter* sp., *Duganella* sp., *Dyella* sp. and *Sphingomonas* sp. as well as those of the Gram-positive bacteria *Pseudomonas* spp., *Vibrio* spp. The *Cytophaga–Flavobacterium–Bacteriodes* phylum, *Coryneforms*, *Arthrobacter* sp. and *Micrococcus* sp. (Gilbert et al. 2004; Amico et al. 2006). Psychrophilic and psychrotolerant aerobic methanotrophic bacteria belonging to the genera and species of *Methylobacter* sp., *Methylosphaera* sp., *Methylocella* sp. and *Methylocapsa acidiphila*, *Methylomonas scandinavica* also inhabits cold ecosystems (Trotsenko and Khmelenina 2005).

In spite of the dominance of the cold environment by bacteria, both in number and density, they are still found in equivalent proportions in the hydrothermal vents together with *Methanogenium* and *Methanococcus* being the most cited genera. In other Antarctic ecosystems, *Oscillatoria*, *Phormidium* and *Nostoc commune* cyanobacteria are said to exercise dominance (Pandey et al. 2004). Heterotrophic bacteria belonging to five major phyla Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes and Deinococcus–Thermus have been recovered from different continental Antarctica and the Antarctic Peninsula (Peeters et al. 2011).

Many cold-active bacterial communities live and thrive in the low-temperature environments of deep sea hydrothermal vent (Yayanos 1995), Antarctic and Arctic sea ice (Tranter et al. 2004), Antarctic subglacial environment, alpine or glacial transitory ponds and ice-covered lakes

Table 1 Some cold-adapted psychrophilic lipase bacteria from a variety of cold environments

Bacteria	Environment	References
Aeromonas sp. LPB 4	Deep-sea sediment	(Lee et al. 2003)
Aeromonas hydrophila	Food products	(Imbert and Gancel 2004)
Arthrobacter globiformis SI55	Recombinant	(Berger et al. 1996)
Acinetobacter baumannii BD5	Mountain water	(Park et al. 2009)
Acinetobacter sp. strain no. 6	Siberian tundra soil	(Suzuki et al. 2001)
Bacillus psychrosaccharolyticus	Soil and lowland marshes	(Seo et al. 2004)
Colwellia psychrerythraea 34H	Arctic marine sea	(Methé et al. 2005; Do et al. 2013)
Desulfotalea psychrophila	Arctic sediments	(Rabus et al. 2004)
Halomonas sp. BRI 8	Antarctic sea	(Jadhav et al. 2013)
Kordia algicida gen. nov., sp. OT-1T	Red tide	(Sohn et al. 2004)
Micrococcus roseus	Glacial soil	(Joseph et al. 2011)
Moraxella TA144	Antarctic sea water	(Feller et al. 1991)
Moritella profunda sp. 2674^{T} *Moritella abyssi* sp. 2693^{T}	Atlantic sediments	(Xu et al. 2003)
Moritella sp 2-5-10-1	Antarctic bacteria	(Wang et al. 2013)
Pelagibacterium halotolerans B2T	East China Sea	(Wei et al. 2013)
Photobacterium strain (MA1-3)	Blood clam	(Kim et al. 2012)
Photobacterium aplysiae sp. (GMD509)	Eggs of sea hare	(Seo et al. 2005b)
Photobacterium frigidiphilum sp. SL13$^{(T)}$	Deep-sea sediment	(Seo et al. 2005a)
Photobacterium lipolyticum sp. M37	Intertidal Yellow Sea	(Yoon et al. 2005; Ryu et al. 2006)
Photobacterium profundum sp. DSJ4	Sediment	(Nogi et al. 1998)
Photobacterium ganghwense sp. FR1311T	Deep-sea	(Park et al. 2006)
Photobacterium marinum AK15$^{(T)}$ and AK18	Sea sediment	(Srinivas et al. 2013)
Photobacterium histaminum sp. JCM 8968	Marine fish/sea water	(Okuzumi et al. 1994)
Pseudoalteromonas haloplanktis TAC125	Marine Antarctica	(Médigue et al. 2005; de Pascale et al. 2008; Russo et al. 2010)
Pseudomonas putida GR12-2	Arctic plant	(Muryoi et al. 2004)
Pseudomonas sp. strain KB700A	Water sample	(Rashid et al. 2001)
Pseudomonas sp. 4	Marine soil/water	(Kavitha and Shanthi 2013)
Pseudomonas antarctica sp	Antarctica	(Reddy et al. 2004)
Pseudomonas. sp strain BII-1	Alaskan soil	(Choo et al. 1998)
Pseudomonas fluorescens	Refrigerated raw milk	(Bucky et al. 1987)
Pseudomonas fluorescens	Soil of cold region	(Leonov 2010)
Pseudomonas sp. strain AMS8	Antarctic soil	(Mohamad Ali et al. 2013)
Pseudomonas sp. strain KB700A	Subterranean sea	(Rashid et al. 2001)
Psychrobacter okhotskensis MD17^{T}	Okhotsk sea coast	(Yumoto et al. 2003)
Psychrobacter cryohalolentis K5^{T}	Siberian cryopeg	(Novototskaya-Vlasova et al. 2013)
Psychrobacter sp 7195.	Deep-sea sediment	(Zhang et al. 2007)
Psychrobacter sp. C18	Deep-sea sediments	(Chen et al. 2010)
Psychrobacter sp. G	Antarctica sea	(Xuezheng et al. 2010)
Psychrobacter sp	Chilean antarctic seawater	(Parra et al. 2008)
Psychrobacter sp. Ant300	Antarctic soil	(Kulakova et al. 2004)
Psychrobacter salsus sp. nov. *Psychrobacter adeliensis* sp. nov	Fast ice Antarctic	(Shivaji et al. 2004)
Pseudoalteromonas sp *Psychrobacter* sp. *Vibrio*. Sp	Antarctic samples	(Lo Giudice et al. 2006)
Shewanella sp. strain SIB1	Water deposits in oil reservoir	(Suzuki et al. 2004)
Staphylococcus epidermidis	Frozen fish	(Joseph et al. 2006)
Stenotrophomonas maltophilia CGMCC 4254	Oil soil	(Li et al. 2013)
Vibrio iliopiscarius sp. Nov *Vibrio salmonicida*	Marine sea Fish	(Onarheim et al. 1994)
Vibrio ruber sp. Nov VR1T	Sea water	(Shieh et al. 2003)

Table 2 Some cold-adapted recombinant lipase secretion, expression conditions, purification strategies and properties

Bacteria	Lipase		Cultivation	Tag	Purification steps	Fold/recovery (%)	MW/pH/temp optima	References
	Intracellular	Extracellular	Temperature					
Acinetobacter baumannii BD5	LipA		37 °C	His-tag	Ni_2 ± Affinity chromatography	n.s.	35 kDa/8.3/35 °C	(Park et al. 2009)
Acinetobacter sp.		No. 6	4 °C	n.s.	80 % acetone precipitation $(NH_4)_2SO_4$/ IEX/HIC	27-fold/61	n.s./20 °C	(Suzuki et al. 2001)
Aeromonas sp.		LPB 4	10 °C	n.s.	Acetone precipitation QAE Sephadex	53.5-fold/7.5	50 kDa/n.s./10 °C	(Lee et al. 2003)
Pseudomonas. sp strain BII-1		lipP	37 °C	n.s.	20–65 % $(NH_4)_2SO_4$ DEAE-cellulose Graphite column	38-fold/17	33 kDa/8.0/45 °C	(Choo et al. 1998b)
Pseudomonas fluorescens KE38		KE38	25 °C	n.s.	$(NH_4)_2SO_4$ Sephadex G-100 Ultrafiltration	41.13-fold/54.99	43 kDa/8.0/20 °C	(Adan Gökbulut and Arslanoğlu 2013)
Pseudomonas fragi IFO3458	rPFL			His-tag	Metal-chelating chromatography	n.s.	32.5 kDa/8.0/29 °C	(Alquati et al. 2002)
Pseudomonas fluorescens		Strain 38	4-10 °C	n.s.	20–60 %$(NH_4)_2SO_4$ Sephadex G-100 Phenyl Sepharose CL-4B	48-fold and 68-fold	155 000 and 175 000	(Bucky et al. 1987)
Pseudomonas sp. strain KB700A	KB-Lip		37 °C	His-tag	HiTrap Q	n.s.	49 kDa/8.5/35 °C	(Rashid et al. 2001)
Pseudomonas sp. 7323	lipA		37 °C	His-tag	Ni–NTA affinity chromatography	14.6-fold/40.3	66 kDa/9/30 °C	(Zhang and Zeng 2008)
Psychrobacter sp. C18	LipX		20 °C	His-tag	Ni2+-Affinity chromatography	10.7-fold/59.1	35 kDa/8.0/30 °C	(Chen et al. 2010)
Psychrobacter sp. Ant300	PsyEst		27 °C	His-tag	His-bind affinity chromatography	n.s./30	43 kDa/35 °C	(Kulakova et al. 2004)
Psychrobacter pacificensis	Est10		22 °C	GST-tag	GST-tag affinity chromatography	n.s.	24.6 kDa/7.5/25 °C	(Wu et al. 2013)
Psychrobacter sp	MBP		20 °C	MBP-tag	Amylose affinity chromatography	n.s.-fold/40	90 kDa/8.0/20 °C	(Parra et al. 2008)
Psychrobacter sp. G	Lip-948		15 °C	His6 tag	Ni-Sepharose FF	n.s./19.8 %	35 kDa/8/35 °C	(Shuo-shuo et al. 2011)
Psychrobacter sp 7195.	LipA1		30 °C	n.s.	DEAE Sepharose CL-4B Sephadex G-75 25–75 %(NH4)2SO4	35-fold/13.7	5 kDa/9.0/30 °C	(Zhang et al. 2007)
Photobacterium sp. MA1-3	MA1-3		18 °C	His-tag	Ni_2 ± nitrilotriacetic	n.s.	39 kDa/8.0/30 °C	(Kim et al. 2013)
Photobacterium lipolyticum	M37		18 °C	His-tag	Ni_2 ± nitrilotriacetic	n.s.	n.s./9.0/25 °C	(Ryu et al. 2006)
Pseudoalteromonas haloplanktis TAC125		Lip 1	4 and 15 °C	n.s.	23 %$(NH_4)_2SO_4$ Q-Sepharose FF	12-fold/7.8	n.s./8.5/40 °C	(de Pascale et al. 2008; Médigue et al. 2005)
Stenotrophomonas maltophilia CGMCC 4254		SML	30 °C	n.s.	Ultraconcentration 60–80 % $(NH_4)_2SO_4$ Phenyl Sepharose	60.5-fold/38.9	52 kDa/8.0/35 °C	(Li et al. 2013)
Streptomyces coelicolor A3(2)	EstC		16 °C	His-tag	IMAC	n.s.	35 kDa/8.5–9/35 °C	(Brault et al. 2012)

n.s.- not specified

(Morgan-kiss et al. 2006), where they represent the most abundant cold-adapted life-forms on earth at the level of species diversity and biomass (Feller and Gerday 2003). Most interesting is the evidence of metabolically active psychrotrophic bacteria in super-cooled, high-altitude cloud droplets (Sattler et al. 2001), surviving subjection to low-temperature, high-pressure, and low-nutrient levels. Considering the potential impact of the biomolecules derived from cold-adapted microorganisms, a great number of microbial biodiversity remains largely unstudied (Kennedy et al. 2008).

Cold-active lipases

Following the discovery of a pancreatic lipase by Claude Bernardin (1856), about 90 % of produced lipases have been obtained from microbial sources (Ellaiah et al. 2004; Kumar and Gupta 2008; Treichel et al. 2010) with many others identified in the environment including, animals and plants (Moussavou Mounguengui et al. 2013). The hunt for new lipases has been on the increase leading to a separate database for true lipases termed lipabase which integrates information about their structural and functional properties including taxonomic and biochemical information (Nagarajan 2012).

Lipases (water-soluble triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes physiologically catalyzing hydrolysis of insoluble long-chain triacylglycerides to free fatty acids (Treichel et al. 2010; Tran et al. 2013). Besides their natural function, they offer considerable potential in catalyzing bioconversion reactions in non-aqueous media and also catalyze hydrolysis of organic carbonates without need for any cofactors (Pandey et al. 1999; Sharma et al. 2001; Dutra et al. 2008; Rajendran et al. 2009). Lipases by convergent evolution are serine hydrolases (Stergiou et al. 2013) and they demonstrate good chemioselectivity, regioselectivity and enatioselectivity. They also possess unique features of being stable even in organic solvents, low thermostability at elevated temperatures with low costs and enantioselective properties (Thakur 2012; Hosseinpour 2012). Available data regarding several lipases from diverse sources indicate alkaline, acidic and organic solvent tolerant and cold-active lipases as most obviously investigated sources for numerous biotechnological applications given the important role they play in the turnover of various organic materials and biomass into useful products under conditions considered unsuitable to other biomolecules (Ramani et al. 2010; Joseph et al. 2011; Yoo et al. 2011; Mander et al. 2012; Nagarajan 2012; de Abreu et al. 2014).

Conventionally, cold-adapted microorganisms synthesize lipases which have evolved to tolerate the extremely inhospitable conditions of cold habitats with high biocatalytic activity. The need to avoid any causes of structural damage makes them irreversibly adapted to these environments by way of evolving unique mechanism to overcome the adverse influence of low temperatures compared to the lipases from mesophiles or thermophiles. Conserving stability–activity–flexibility relationship is fundamental to their functional and structural adaptive properties (Margesin et al. 2007; Buzzini et al. 2012; Feller 2013). Lowered thermal stability of cold-active lipases allows an equilibrium shift during reactions, because effectively functioning at low temperatures guarantees a promising potential for utilizing lipases in 'White Biotechnology' and other industrial applications (Gerday et al. 2000; Hasan et al. 2006; Joseph et al. 2008; Adan Gökbulut and Arslanoğlu 2013).

Cold-adapted lipase are said to offer economic incentives through low energy cost achieved through reduction in heating steps required to function in cold environments. Providing increased reaction yields and minimizing undesirable reactions that can occur at high temperatures by heat-inactivation of the enzymes rather than by use of chemical extraction offers significant advantages to the food industry preventing any modification to the substrates and finished product. Several psychrophlic and psychotrophic bacteria have been exploited for the production of a variety of cold-active lipases across different cold habitats, although current research trends on these classes of enzymes have shown that attention is given to using recombinant and protein engineering strategies to generate strains with promising properties. Few cold-adapted lipase/esterase have been studied. These include the enzymes from *Aeromonas* sp. LPB4 (Lee et al. 2003), *Pseudomonas* sp. strain B11-1 (Choo et al. 1998b), *Acinetobacter* sp. No. 6 (Suzuki et al. 2001), *Psychrobacter* sp. Ant300 (Kulakova et al. 2004), *Photobacterium* sp. (Ryu et al. 2006). Gene encoding a cold-active lipase from Antarctic psychrotrophs *Penicillium expansum* SM3 (Mohammed et al. 2013) *Moraxella* TA144 (Feller et al. 1991) revealed a novel cold-active lipase. Cold-active lipase from *Micrococcus roseus* exhibited high activity and stability over a range of temperature regimes in an optimized semisolid state fermentation utilizing agro-industrial substrates (Joseph et al. 2011). Cloned gene (lipA1) of a cold-active lipase has been reported for a *Psychrobacter* sp. 7195 isolated from a deep-sea sediment (Zhang et al. 2007). Novel cold-active and organic solvent-tolerant lipase displaying remarkable stability have been reported for *Stenotrophomonas maltophilia* CGMCC 4254 isolated from oil-contaminated soil samples (Li et al. 2013). Soils from Alaskan cold habitat and other cold regions have been exploited as potential sources of novel cold-active lipase (Choo et al. 1998a; Leonov 2010). Characterization of cold-active lipase from cold-adapted bacteria from snow-covered soil, salmon intestine and crab intestine have been predicated upon the thermodynamic shifts in temperatures and reductions in enthalpy energy values (Morita

et al. 1997). Culture-independent approach have been applied in constructing a metagenomic library from the unculturable component of microbial communities from various deep-sea sediments, terrestrial environmental niches and other cold habitats towards producing novel cold-active lipase genes from recombinant clones (Kennedy et al. 2008; Jeon et al. 2009).

Structure and cold adaptation in lipases

Knowledge of the relationship between extreme environments and their psychrophilic host requires several integrative attempts. Just as studies on cold-active lipases revolves around their isolation, purification characterization so does elucidation of their molecular and functional characteristics provide a better understanding of these enzymes. Strategy towards structural adaptation is a property unique to each enzyme. The thermodynamic stability of psychrophilic lipases is a strategy characterized by the relationship between stability, conformational flexibility or plasticity and their catalytic efficiency conferred upon them by a range of structural systems, useful especially when compared to mesophilic and thermophilic enzymes operating at set conditions of low temperatures. Rapid engineering paradigms involving gene synthesis, cloning and overexpression systems (Emond et al. 2010; Chen et al. 2010; Novototskaya-Vlasova et al. 2013), modular regulation (Palomo et al. 2003; Reetz et al. 2010), X-crystallography (Uppenberg et al. 1995), modification (Juhl et al. 2010; Durmaz et al. 2013), rational modeling (Alquati et al. 2002; Mohamad Ali et al. 2013; Maraite et al. 2013) have proven useful for understanding the structural adaptation of several cloned cold-active lipase.

Array of new methods enabling investigations into the structural and molecular parameters of cold-active lipases has been developed (Parra et al. 2008; Jeon et al. 2009; Do et al. 2013). Cold-active lipolytic genes encoding for two different lipases (*Lip-1452* and *Lip-948*) from an Antarctic *Psychrobacter* sp. were successfully cloned and expressed with a description of their primary structure given (Xuezheng et al. 2010). A recombinant fusion protein (MBP-lipase) from an Antarctic marine *Psychrobacter* sp. has been cloned and characterized with significant cold-adapted features and activity (Parra et al. 2008). Comparative studies of the three-dimensional model of a recombinant lipase from *Pseudomonas fragi* IFO 3458 (PFL) with the structures of mesophilic homologous lipases were shown to account for PFL activity at low temperature (Alquati et al. 2002). This property is conferred by synergistic changes in overall genome content and protein sequence as revealed in the works of Methé et al. (2005) wherein modeling of three-dimensional *Colwellia psychrerythraea* and genome protein homology suggests changes to proteome composition that may enhance enzyme adaptation at low temperatures. The tendency for obtaining psychrophilic enzymes from a mesophilic and thermophilic counterparts could also be deduced in a homologous substitution of charged residues of Arg and Gluwith Lys and Ala, respectively (Gianese et al. 2001). Reduction in arginine/lysine residues is often less uniformly distributed in psychrophilic than in mesophilic enzymes. A few charged surface residues are involved in stabilizing intramolecular salt bridges and a large proportion of them exposed at the protein surface enable increased solvent interaction and enhance conformational flexibility accompanied by increased thermolability (Alquati et al. 2002; Siddiqui and Cavicchioli 2006; Feller 2013). Another important adaptive response to cold temperature below 0 °C comes from the production of trehalose and exopolysaccharides (EPSs) playing multiple roles in the entrapment, adhesion, retention and survival of microorganisms. It also favors the sequestration and concentration of nutrients, protection against cold shocks damage by modification of the physicochemical environment around the bacterial cell, acting as buffers and cryoprotectant in the prevention of denaturation and precipitation of proteins (Krembs et al. 2002; Ewert and Deming 2013).

Cold-active lipase expression and regulation

Overexpression and secretion of soluble proteins allows for their projected application in finding functional and structural information of large numbers of proteins. The induction of gene expression at sub-optimal growth temperatures also improves the solubility of proteins which is sometimes a major bottleneck hampering heterologous protein production in the periplasm of most microbial host. The synthesis and secretion of lipases by bacteria is influenced by a variety of factors. While rapid protein expression often results in unfolded/misfolded proteins, the reduced environment of the bacterial cytosol and the inability of host cell such as *E. coli* to perform several eukaryotic post-translational modifications could result in the insoluble expression of proteins (Francis and Rebecca 2010).

Strategies for recombinant production and expression of cold-adapted lipases and other lipase enzymes, development of alternative promoter and induction strategies, modification of the host cells by engineering strategies and shifting the growth and manipulating the expression conditions with reduction in post-induction temperature (Weickert et al. 1996) has offered the options crucial to abundant yield towards efficient production of a diverse range of soluble heterologous proteins as well as regulating the cytotoxic effect of their host cells. Variety of expression vectors with different affinity tag sequences has been designed for fusion to almost any target protein that can be cloned and expressed in a microbial host. Each of these hosts are also equipped with an N-terminal signal sequence so that they

can traverse the cytoplasmic membrane (Schlegel et al. 2013), making them suitable for certain recombinant protein purification procedures. More so, cleavage sites engineered between the affinity tag and the protein of choice enables removal of these tags (Einhauer and Jungbauer 2001). Higher yields of protein obtained with affinity tag makes this alternative economically favorable (Arnau et al. 2006). Thorough consideration is needed especially where the possibility of an adverse effect of the fusion on the applicability to the host system of choice could affect proper protein expression and secretion. A possible strategy is the co-expression of the recombinant protein with molecular chaperone. By employing a combination of functional metagenomic and protein expression technology approach, Jeon et al. (2011) successfully obtained higher yield of purified positive lipase producing clones with cold-activity property. Conversely, they demonstrated that the combination of co-expression of chaperones, removal of signal sequence and induction at low temperature (16 °C) may be effective in regulating soluble expression of lipolytic enzyme-encoding genes. Sometimes expression at low temperature alleviates toxicity, but may often lead to only partial stabilization of the fusion protein (Mujacic et al. 1999). Similar observations were highlighted by Shuo-shuo et al. (2011) wherein a cold-active lipase gene *Lip-948*, cloned from Antarctic psychrotrophic bacterium (*Psychrobacter* sp. G) showed enhanced intracellular soluble protein expression when co-expressed with "chaperone consortium" plasmids at a cultivation temperature of 15 °C. Novototskaya-Vlasova et al. (2013) described the influence of multiple parameters as folding enhancers when they developed an efficient protocol for solubilization and subsequent refolding of recombinant *Lip2Pc* lipase from *Psychrobacter cryohalolentis* K5T in the presence of a truncated chaperone. Rashid et al. (2001) described the production of low-temperature lipase both intracellularly and extracellularly from a previously reported psychrotrophic bacterium *Pseudomonas* sp. strain KB700A, which displays sigmoidal growth even at −5 °C. Although the genes were expressed under various conditions, the protein product was consistently produced in an insoluble form as inclusion bodies. However, treatment of insoluble recombinant lipase with urea and refolding by fractional dialysis enhanced purity and homogeneity. Similarly, expression of some lipase have only been achieved in insoluble form necessitating further refolding strategies as reported for a *BDLipA* cold-adapted lipase produced by *Acinetobacter baumannii* BD5 cloned and co-expressed in *Escherichia coli* BL21 (trxB) with a lipase chaperone as an inclusion body (Park et al. 2009). Overproduction, expression and optimization of *LipXHis* lipase from a psychrophilic deep-sea sediment *Psychrobacter* sp. strain C18 was achieved by amplifying the *lipX* lipase encoding gene without a signal peptide (Chen et al. 2010). In *Escherichia coli*, some proteins and location of secretion signal have been shown to enhance and promote efficient secretion of lipase to the extracellular medium. Thus, some lipases from *Pseudomonas fluorescens* have been described to secrete lipase through the signal peptide-independent pathway (Duong et al. 1994). Co-expression of *rPFL* with the foldase of *P. aeruginosa* as observed by Alquati et al. (2002) did not produce significant improvements in the fraction of soluble lipase, apparently suggesting the hypothesis that *rPFL* might also be secreted by a signal peptide-independent pathway. A sequence alignment of a novel *M37* lipase investigated by Ryu et al. (2006) reveals no signal sequence and the resulting protein when cultivated and induced at 37 and 18 °C was exclusively insoluble and soluble, respectively. However, it was hypothesized that *M37* lipase might be another example of extracellular proteins secreted without signal sequence. *LipA* protein was expressed as a His-tagged fusion protein in E. coli and successfully secreted extracellularly by OmpA secretion signals at the N-terminal regions (Zhang and Zeng 2008).

Mutant host of *E. coli* (C41(DE3) and C43(DE3) have also been reported to give high saturation cell density, and produced the protein as inclusion bodies at an elevated level without toxic effect. Mutants are frequently used to overcome the toxicity associated with overexpressing recombinant proteins using the bacteriophage T7 RNA polymerase expression system, even when the toxicity of the plasmids is so high that it prevents transformation in the strain (Dumon-Seignovert et al. 2004).

Although several reports have documented efficient secretion and folding of active lipase (Jaeger et al. 1999), over the years efforts have been made towards optimizing protein secretion and expression with several focus on strategies designed to maximize the yields of recombinant proteins and major challenges facing the use of prokaryotic expression system (Hannig and Makrides 1998; Francis and Rebecca 2010).

Purification approaches for cold-active lipases

The Antarctic marine habitats are unique natural laboratories for major research on the evolution of cold-active lipases in extreme environments (Russo et al. 2010). It has become evidently clear that obtaining cold-active lipase from microbial host of these extreme environments does not just stop at isolation, identification and screening/production. However, homogeneity and purity are the currency for achieving their unique purpose as biocatalysts for various industrial and biotechnological applications. Conversely, purity enables the molecular characteristics of cold-active lipases originating from Antarctic bacteria in relation to the adaptation to cold, to acquire high catalytic

efficiency at low temperature, enhanced stability and higher flexibility (Nagarajan 2012). A major challenge with cold-active lipases has been obtaining their purified forms, where lipopolysaccharides bonding with lipid hydrolases in the Antarctic microorganisms are difficult to break (Gerday et al. 1997). Several traditional and novel purification and optimization strategies have been developed and employed successfully (Yujun et al. 2008; Basheer and Thenmozhi 2010). Precipitation steps are usually followed by chromatographic steps like gel filtration and affinity chromatography. Following these two is the hydrophobic interaction chromatography (HIC) (Nagarajan 2012). With respect to the desired purity of lipases, different steps from pre- to post-purification periods have involved the use of both physical and molecular technologies, respectively. Ammonium sulfate purification and organic solvent extraction have been used for certain applications just as Iftikhar et al. (2011) have also described a successful purification of lipases of up to 100 % with 710.02-fold. Economically, certain applications of lipases might not be achieved with just the usual procedures of precipitation steps, therefore further purification options are necessary (Gupta et al. 2004).

Industrial potential of cold-adapted lipase

Lipases are adaptable and increasing attention has been drawn to potential applications of psychrophilic lipases from microorganisms populating permanently cold environments (Pfeffer et al. 2007; Długołecka et al. 2008). Some biotechnological and industrial potential of cold-active lipases include the following.

Detergent additives

Lower washing temperature, improved energy conservation and minimization in wear and tear are obvious essential benefits of cold-active lipases in the detergent industries (Gerday et al. 2000). These enzymes have proven useful for cleaning applications that have potential to extend their effectiveness in enzyme-based, low-temperature cleaning formulations for laundry and dishwashers, reducing environmental burden and enabling the biodegradation of undesirable chemicals in detergents, leaving no harmful residues while ensuring environmental sustainability (Joseph et al. 2008). The ability of enzymes to clean effectively in detergents at low temperature has seen a reduction in temperature used for washing procedures in a range of industries. Cold-active lipase have been used as detergent formulations on porous building materials that ordinarily cannot be immersed or moved into cleaning solutions and other mold-infested surfaces reducing the damage normally associated with the use of standard cleaning agents (Valentini et al. 2010). Lipase preparations active at low and ambient temperatures in detergent removes oil stains by decomposing them into more hydrophilic substances while also maintaining the texture and quality of fabrics (Fujii et al. 1986; Weerasoriya and Kumarasinghe 2012).

Textile industry

Successive bio-washing and stone washing of fabric materials in the textile industries often reduce the smoothness of the tissues constituting the main fibers. Pretreatment with cold-adapted lipase reduces the pill-formation and increases the durability and softness of the tissue. They lower the temperature of the process conditions in textile industries. Mechanical resistance of final fabric quality is greatly enhanced as a result of the spontaneous rapid inactivation of cold-adapted enzymes at higher temperature. Enzymatic desizing of materials in fabric has advantages over the traditional process, which uses acid or oxidizing agents. For this purpose, bacterial lipases from *Pseudomonas cepacia, Pseudomonas fragi, Pseudomonas fluorescens, Pseudomonas stutzeri* has been used (Hasan et al. 2006).

Environmental bioremediation

Microorganisms have been efficiently used in the bioremediation and low molecular wastewater treatment in cold climates and in lowering the amount of toxic compounds previously considered non-degradable (Margesin and Feller 2010). Cold-adapted lipases are potentially applicable as bioremediation agents in wastewater treatment, in situ bioremediation of fat-contaminated cold environment, synthesis of organic compounds and lowering of toxic compounds in the environment such as nitrates, hydrocarbons, aromatic compounds, heavy metals and biopolymers such as cellulase, chitin, lignin, proteins and triacylglycerols (Wakelin and Forster 1997; Margesin and Schinner 1997, 1998; Timmis and Pieper 1999; van Langen et al. 1999; Suzuki et al. 2001; Margesin 2007). Strains preparations with applicable potential in the bioremediation of oil-polluted sites under the conditions of a cold climate have been investigated (Belousova and Shkidchenko 2004; Amara and Salem 2009).

Food industry

Maintaining the ambient temperature of food is a major way of preventing spoilage and deterioration and of minimizing undesirable changes in chemical and physical qualities of food which ordinarily could occur under high-temperature storage conditions. The relevance of cold-active lipase to the food and feed industry most importantly prevents spoilage and undesirable changes in nutritional compositions of most

heat-sensitive substrates utilized in food processing (Russell 1998; Gerday et al. 2000; Cavicchioli et al. 2002). Despite their gains to the modern food industry, cold-adapted microorganism have also been implicated in the spoilage of refrigerated meat and raw milk, affecting the quality and shelf-life, thus making these foods unacceptable to consumers (Dieckelmann et al. 1998; Abdou 2003; Samaržija et al. 2012). Psychrotroph-derived lipases have also been applied in the non-aqueous synthesis of a model ester (butyl caprylate). Studies have revealed distinguishing characteristics of cold-active lipases with a strong potential for the organic synthesis of valuable short-chain esters such as flavors used in food and pharmaceuticals (Brault et al. 2012; Li et al. 2013).

Medical and pharmaceutical applications

Chiral intermediates and fine chemicals are in high demand both by pharmaceutical and agrochemical industries, for the preparation of bulk drug substances and agricultural products (Patel 2002). Synthesis and modification of optically pure chiral drugs have been well documented for lipases years ago (Margolin 1993) and recent advances in the synthesis of optically pure compounds have embraced biocatalytic procedures using lipases for the preparation of chiral pharmaceuticals, which offer a clean and ecological way of performing chemical processes in mild reaction conditions and with high degree of selectivity and these are playing an increasingly prominent role (Margolin 1993; Gotor-Fernández et al. 2006a). Simplicity of use, low cost, commercial availability and recycling possibility makes cold-active lipase ideal for the synthesis and resolution of a wide range of nitrogenated compounds in drug synthesis (Gotor-Fernández et al. 2006b).

Low water biocatalysis

Cold temperatures affect the dynamic activity of bulk water as well as the spheres of hydration surrounding the protein surface. The hydration energies of cold-active enzymes are generally less affected by lower temperatures, and their inherent lower surface hydrophobicity is less sensitive than mesophilic proteins, keeping their structures more intact (Fields 2001; Zhong et al. 2011).

Conclusion

Generally, lipase enzymes offer economic benefits in their industrial and biotechnological applications globally. Cold-adapted microorganisms have become potential targets for lipases exploited for numerous biotechnological gains based on their ability to withstand prevailing challenges of permanently cold habitats. Cold-active lipases represent a versatile group of bacterial extracellular enzymes. With adaptive-response strategies, the extreme environments have now become accessible to these cold-adapted psychrophilic enzymes. Researches on the extreme adaptations of cold-adapted microorganisms have stirred up keen interest among several research groups and R&D investment; and several hundreds of researches are ongoing to shed new light on other characteristics of these fascinating organisms that will be of applicable potential benefits.

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