Biotechnology and Molecular Biology Review Vol. 2 (2), pp. 039-048, June 2007
Available online at http://www.academicjournals.org/BMBR
ISSN 1538-2273 © 2007 Academic Journals

Standard Review

Standard Review Cold-active microbial Lipases: a versatile tool for industrial applications

Babu Joseph[1]*, Pramod W. Ramteke[1], George Thomas[2], and Nitisha Shrivastava[1]

[1]Department of Microbiology and Microbial Technology, College of Biotechnology and Allied Sciences, Allahabad Agricultural Institute- Deemed University, Allahabad 211 007, Uttar Pradesh, India.
[2]Department of Molecular Biology and Genetic Engineering, College of Biotechnology and Allied Sciences, Allahabad Agricultural Institute- Deemed University, Allahabad 211 007, Uttar Pradesh, India.

Accepted 11 May, 2007

Lipases are a class of enzymes which catalyze the hydrolysis of long chain triglycerides and constitute the most important group of biocatalysts for biotechnological applications. Cold active lipases have lately attracted attention as a result of their increasing use in the organic synthesis of chiral intermediates. Due to their low optimum temperature and high activity at very low temperatures, which are favorable properties for the production of relatively frail compounds. Cold active lipases are today the enzymes of choice for organic chemists, pharmacists, biophysicists, biochemical and process engineers, biotechnologists, microbiologists and biochemists. The present review describes various industrial applications of cold active microbial lipases in the medical and pharmaceuticals, fine chemical synthesis, food Industry, domestic and environmental applications.

Key words: Biocatalysts, cold active lipase, enzymes, industrial application, lipolytic, Psychrophiles.

Table of Contents

INTRODUCTION

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential (Benjamin and Pandey, 1998). They constitute the most important group of biocatalysts for biotechnological applications. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agro-chemicals, and flavour compounds (Jaeger and Eggert, 2002). The chemo-, regio- and enantio-specific behavior of these enzymes caused tremendous interest among scientists and Industrialists (Saxena et al., 2003). The knowledge of cold adapted lipolytic enzymes in industrial applications is increasing at a rapid and exciting rate. Unfortunately, the studies on cold adapted lipases are incomplete and scattered. Till date no attempt has been undertaken to organize this information. Hence, an over- of this biotech-

*Corresponding author. E-mail: babuaaidu@yahoo.co.in. Tel: +91 532 2684296. Fax: +91 532 2684593

Table 1. Bacteria producing cold adapted lipases.

Microorganism	Sources	Reference
Acinetobacter sp. strain No. 6	Siberian tundra soil	Suzuki et al., 2001
Acinetobacter sp. strain No. O_{16}	Ns	Breuil and Kushner, 1975
Achromobacter lipolyticum	Ns	Khan et al., 1967
Aeromonas sp. strain No. LPB 4	Sea sediments	Lee et al., 2003
Aeromonas hydrophila	Marine habitat	Pemberton et al., 1997
Bacillus sphaericus MTCC 7526	Gangothri Glacier (Western Himalayas)	Joseph, 2006
Microbacterium phyllosphaerae MTCC 7530		
Moraxella sp.	Antarctic habitat	Feller et al., 1990
Morexella sp TA144	Antarctic habitat	Feller et al., 1991
Photobacterium lipolyticum M37	Marine habitat	Ryu et al., 2006
Pseudoalteromonas sp. wp27	Deep sea sediments	Zeng et al., 2004
Pseudoalteromonas sp.	Antarctic marine	Giudice et al., 2006
Psychrobacter sp.		
Vibrio sp.		
Pseudomonas sp. strain KB700A	Subterranean environment	Rashid et al., 2001
Pseudomonas sp. B11-1:	Alaskan soil	Choo et al., 1998
Pseudomonas P38	NS	Tan et al., 1996
Pseudomonas fluorescens	Refrigerated milk samples	Dieckelmann et al., 1998
Pseudomonas fluorescens	Refrigerated food	Andersson 1980
Pseudomonas fluorescens	Refrigerated human placental extracts	Preuss et al., 2001
Pseudomonas fragi strain no. IFO3458	BCCM^{TM}/LMG2191^{T} Bacteria collection, Universiteit Gent, Belgium	Alquati et al., 2002
Pseudomonas fragi Strain no. IFO 12049	Ns	Aoyama et al., 1988
Psychrobacter sp. wp37	Deep sea sediments	Zeng et al., 2004
Psychrobacter okhotskensis sp.	Sea coast	Yumoto et al., 2003
Psychrobacter sp. Ant300	Antarctic habitat	Kulakovaa et al., 2004
Psychrobacter immobilis strain B 10	Antarctic habitat	Arpigny et al., 1997
Serratia marcescens	Raw milk	Abdou, 2003
Staphylococcus aureus	Ns	Alford and Pierce, 1961
Staphylococcus epidermidis	Frozen fish samples	Joseph et al., 2006

Ns Not specified.

nologically and industrially important enzyme and its applications has been collected and compiled from the information available in the literature. From the limited number of available reports on cold active lipases, it is clear that most of the studies were focused on isolation, purification and characterization for industrial applications of these enzymes followed by gene cloning, expression and sequencing. A worldwide initiative has been taken up for exploring cold active lipase producing microorganisms and their industrial applications.

Sources of cold active lipases

Cold adapted lipases are largely distributed in microorganisms existing at low temperatures nearly 5°C. Although a number of lipase producing sources are available, only a few bacteria and yeast were exploited for the production of cold adapted lipases (Joseph, 2006). Attempts have been made from time to time to isolate cold adapted lipases from these microorganisms having high activity at low temperatures. A list of various cold adapted lipase producing psychrophillic and psychro-trophic bacteria are presented in Table 1.

Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to the absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer (Wiseman, 1995). Various studies showed that a high bacterial count has been recorded as high as 10^5 and 10^6 ml^{-1} in water column and in the sea ice respectively (Sulivan and Palmisano, 1984; Delille, 1993). Cold adapted bacterial strains were isolated mostly from Antarctic and Polar regions, which represents a permanently cold (0 ± 2°C) and constant temperature habitat. Another potential source of cold active lipases is deep sea bacteria. A marine bacterium *Aeromonas hydrophila* growing at a temperature range between 4 and 37°C was found to produce lipolytic enzyme (Pemberton et al., 1997).

Table 2. Fungi producing cold adapted lipases.

Microorganism	Sources	Reference
Aspergillus nidulans	Ns	Mayordomo et al., 2000
Candida antarctica	Antarctic habitat	Patkar et al., 1993; Uppenberg et al., 1994a; Uppenberg et al., 1994b Patkar et al., 1997 Koops et al.,1999; Zhang et al., 2003; Siddiqui and Cavicchioli, 2005
C. lipolytica	Frozen food	Alford and Pierce, 1961
Geotrichum candidum	Frozen food	Alford and Pierce, 1961
Pencillium roqueforti	Frozen food	Alford and Pierce, 1961
Rhizopus sp.	Frozen food	Coenen et al., 1997
Mucor sp.	Frozen food	Coenen et al., 1997

Ns - Not specified

However, 16S rDNA sequence of isolated cold adapted *A. hydrophila* exhibited the highest similarity to that of a marine bacterium *A. hydrophila* (95% homology) and showed the same characteristics. This isolate could grow at temperature 4, 10, 20, and 30°C but not at temperature above 30°C (Lee et al., 2003). Few bacterial genera have been isolated and characterized from deep-sea sediments where temperature is below 3°C. They include *Aeromonas* sp. (Lee et al., 2003); *Pseudoalteromonas* sp. and *Psychrobacter* sp. (Zeng et al., 2004); *Photobacterium lipolyticum* (Ryu et al., 2006). Permanently cold regions such as glaciers and mountain regions are another habitat for psychroplillic lipase producing microorganisms (Joseph, 2006). The soil and ice in Alpine region also harbor psychrophillic microorganisms, which produces cold active lipases. In addition to all these permanently cold regions, there are many other accessible and visible soil and water, which become cold both diurnally and seasonally from which cold active lipase producing microbes can be isolated using appropriate low temperature techniques.

The wide spread use of refrigeration to store fresh and preserved foodstuffs provides a great diversity of nutrient rich habitat for some well known psychrotolerant food spoilage microorganisms. Bacterial genera including *Pseudomonas fragi* (Aoyama et al., 1988; Alquati et al., 2002), *Pseudomonas fluorescens* (Dieckelmann et al., 1998) and *Serratia marcences* (Abdou, 2003), which produce cold active lipases, were isolated from refrigerated milk and food samples. Further, the genes encoding for cold active lipases were isolated and cloned into mesophilic bacteria (*Escherichia coli*) as host organism and used for their expression. However, the review of Gerday et al. (1997) revealed an extremely unstable condition for the expression of cold adapted lipases within their host (Feller et al., 1990; Feller et al., 1991). In the other reports related to expression studies, the stability of gene encoding lipase production in the host is not clear.

Even though many psychrophilic and psychrotrophic bacteria produce lipases, it is clear that only a few lipolytic fungus was reported to produce cold active lipases (Table 2). An extensive research has been carried out in the cold active lipase of *Candida. antarctica* compared to the other psychrophillic fungi. *Candida lipolytica, Geotrichum candidum* and *Pencillium roqueforti* have also been isolated from frozen food samples and reported to produce cold active lipases (Alford and Pierce, 1961). Psychrotropic lipolytic moulds viz., *Rhizopus* sp. and *Mucor* sp. cause havoc with milk and dairy products and soft fruits (Coenen et al., 1997).

Structural modifications for cold activity

Cold adapted lipases probably are structurally modified by an increasing flexibility of the polypeptide chain enabling an easier accommodation of substrates at low temperature. The fundamental issues concerning molecular basis of cold activity and the interplay between flexibility and catalytic efficiency are of important in the study of structure-function relationships in enzymes.

Such issues are often approached through comparison with the mesophilic or thermophilic counterparts, by site directed mutagenesis and 3D crystal structures (Narinx et al., 1997; Wintrode et al., 2000). The molecular modeling of *Pseudomonas immobilis* lipase revealed several features of cold-adapted lipases (Arpigny et al., 1997). A very low proportion of arginine residues as compared to lysine, a low content in proline residues, a small hydrophobic core, a very small number of salt bridges and of aromatic-aromatic interactions are the possible features of lipase for cold adaptation. Similarly the weakening of hydrophobic clusters, the dramatic decrease (40%) of the

Proline content and of the ratio Arg/Arg+Lys makes lipases active at low temperature (Gerday et al., 1997). Moreover when compared to the dehalogenase from *Xanthobacter autotrophicus*, the cold lipase displays a very small number of aromatic – aromatic interactions and of salt bridges. The location of some salt bridges which are absent in the cold lipase seems to be crucial for the adaptation to cold. A large amount of charged residues exposed at the protein surface, have been detected in the cold active lipase from *Pseudomonas fragi* (Alquati et al., 2002). They also observed a reduced number of disulphide bridges and of prolines in loop structures. Arginine residues were distributed differently than in mesophilic enzymes, with only a few residues involved in stabilizing intramolecular salt bridges and a large proportion of them exposed at the protein surface that may contribute to increased conformational flexibility of the cold-active lipase. In addition to this, the structural factors possibly involved in cold adaption are increased number and clustering of glycine residues (providing local mobility), lower number of ion pairs and weakening of charge-dipole interactions in α helices (Georlette et al., 2004; Gomes and Steiner, 2004). The substitution of Glycine with proline by mutation caused a shift of the acyl chain length specificity of the enzyme towards short chain fatty acid esters and enhanced themostability of the enzyme (Kulakovaa et al., 2004). A mutation in the lid region of catalytic triad of cold active lipases from *P. fragi* improved substrate selectivity and thermostability (Santarossa et al., 2005). Introduction of polar residues in the surface exposed lid might be involved in improved substrate specificity and protein flexibility. The sequence alignment study of cold active lipase from *P. lipolyticum* showed three amino acid residues (Ser174, Asp236 and His312) constitute the active site and RG residues (Arg236 and Gly91) making an oxyanion sequence (Ryu et al., 2006). It is understood that the catalytic cavity of the psychrophillic lipase is characterized by high plasticity. These structural adaptations may confer on the enzyme a more flexible structure, in accord with its low activation energy and its low thermal stability. The above discussions may help to obtain information for insights into the molecular mechanisms of cold adaptation and thermolability of cold active lipases and low thermostability and unusual properties like chemo-, regio- and enantiospecificities.

Applications of cold active lipases

The ability of psychrophilic enzymes to catalyze reactions at low or moderate temperature offers a great industrial and biotechnological potential (Gomes and Steiner, 2004). The 'cold activity' (that is, high catalytic activity at low temperatures) and thermolability of lipases might be the key to success in some of their applications. These applications include their use as catalyst for organic synthesis of unstable compounds at low temperature.

Efficient binding of substrate by the enzyme is mediated by the nature and strength of weak interactions which are of two types. Interactions formed with a negative modification of enthalpy and hence are exothermic (Van der Waals interactions, Hydrogen bonds, electrostatic inter-actions) and interactions formed (at least within the low and moderate temperature ranges) with the positive, modification of enthalpy, and thus are endothermic (hydrophobic interactions). The former destabilized by an increase of temperature, whereas the later will tend to be stabilized by moderately high temperatures (Georlette et al., 2004). The cold active lipases from cold adapted microorganisms and their potential applications have been examined (Choo et al., 1998). With the increasing interest in psychrophiles (microorganisms growing at 0˚C or lower sometimes restricted to organisms that cannot grow above 20˚C) and their applications, cold active lipases will represent a larger share of industrial enzyme market in the coming years. The cold active lipases offer novel opportunities for biotechnological exploitation based on their high catalytic activity at low temperature.

The cold enzymes along with the producing microorganisms cover a broad spectrum of biotechnological applications (Table 3). They include additives in deter-gents (cold washing), additives in food industries (fermentation, cheese manufacture, bakery, meat tenderizing), environmental bioremediations (digesters, composting, oil degradation or xenobiotic biology applications and molecular biology applications), bio-transformation and heterologous gene expression in psychrophilic hosts to prevent formation of inclusion bodies (Feller et al., 1996). A number of relatively straightforward reasons for applications of cold active enzymes in biotechnology have been mentioned by various authors (Rusell, 1998; Margesin and Schinner, 1999; Ohigiya et al., 1999; Gerday et al., 2000; Cavicchioli et al., 2002). Cold active lipases have low thermal stability favorable for some purposes eg: heat liabile lipase can be inactivated by treatment for short periods of time at relatively low temperatures after being used for the processing of food and other materials (Margesin et al., 2002). Thus materials can be prevented from damage during heat inactivation. However, the number of present uses is limited and likely to reflect the state of the field, which has not yet developed as rapidly when compared to the field of thermophilic enzymes. Nevertheless, despite the difficulties with predication, some important advances have been made (Cui et al., 1999). Cold active lipases could be a good alternative to mesophilic enzymes in brewing industry and wine Industries, cheese manufacturing, animal feed supplements, and so on (Collins et al., 2002). The biotechnological potential of cold adapted lipases is in protein polymerization and gelling in fish flesh, improvement in food texture, perfumery and optically active ester synthesis (Cavicchioli and Siddiqui, 2004).

Table 3. Biotechnological applications of cold adapted lipases

Field of Application	Purpose	Reference
Medical and Pharmaceutical application	Synthesis of Aryl aliphatic glycolipids	Otto et al., 2000
	Ethyl esterification of docosahexaenoic acid to Ethyl docosahexaenoate (EtDHA)	Shimada et al., 2001
	Synthesis of citronellol laurate from citronellol and lauric acid	Ganapati and Piyush, 2005
Fine chemical synthesis	Optically active ester synthesis	Anderson et al., 1998
	Ester synthesis, desymmetrization and production of peracids	Zhang et al., 2003
	Organic synthesis of chiral intermediates	Gerday et al., 2000
	Synthesis of butyl caprylate in n-heptane	Tan et al. 1996
	Synthesis of butyl lactate by transesterification	Pirozzi and Greco, 2004
	synthesis of amides	Slotema et al., 2003
Food Industry	Protein polymerization and gelling in fish flesh, improvement in food texture, flavor modification	Cavicchioli and Siddiqui, 2004
	Production of fatty acids and interesterification of fats	Jaeger and Eggert, 2002
Domestic application	Detergents and cold water washing	Gerday et al., 2000
		Joseph, 2006
	Production of α-butylglucoside lactate by transesterification for cosmetics	Bousquet et al., 1999
	Conversion of degummed soybean oil to biodiesel fuel	Watanabe et al., 2002
	Synthesis of lipase-catalyzed biodiesel	Chang et al., 2004
Environmental application	Degradation of lipid wastes	Ramteke et al., 2005
	Bioremediation and bioaugumentation	Gerday et al., 2000; Suzuki et al., 2001; Lee et al., 2003
	Removal of solid and water pollution by hydrocarbons, oils and lipids	Margesin et al., 2002

Medical and pharmaceutical application

Enantioselective interesterification and transesterification have great significance in pharmaceutical for selective acylation and deacylation (Stinson, 1995). Lipases are important in application in pharmaceuticals in transesterificatrion and hydrolysis reaction. They play a prime role in production of specialty lipids and digestive aids (Vulfson, 1994). The alteration of temperature during the esterification reaction drastically changes the enantiomeric values and also the stereopreference (Yasufuku et al., 1996). Lipases play an important role in modification of monoglycerides for use as emulsifiers in pharmaceutical applications (Sharma et al., 2001).

Psychrophiles producing cold active lipases may be a good source for polyunsaturated fatty acids for the pharmaceutical industry. It is because of their excellent capability for specific regioselective reactions in a variety of organic solvents with broad substrate recognition makes lipases as an important biocatalyst in biomedical applications (Margesin et al., 2002). A preparation of optically active amines that was intermediate in the preparation of pharmaceuticals and pesticides, which involved in reacting stereospecific N- acylamines with lipases, preferably from *Candida antarctica* or *Pseudomonas* sp. (Smidt et al., 1996). In an attempt to determine the substrate specificity for lipases, alkyl esters of 2-arypropionic acids, a class of non-steroidal anti-inflammatory drugs, were hydrolyzed with *Caenorhabditis rugosa* lipase in which all transformations were highly enantioselective (Botta et al., 1997).

Synthesis of fine chemicals

Some of the industrially important chemicals manufactured from fats and oils by chemical processes could be produced by lipases with greater rapidity and better specificity under mild conditions (Sih and Wu, 1989; Vulfson, 1994). The use of industrial enzymes allows the technologists to develop processes that more closely approach the gentle, efficient process in nature. Some of these processes using cold active lipase from *C. antarctica* have been patented by pharmaceutical, chemical and food industries. *C. antarctica,* one of 154 species of the genus *Candida* belongs to the phylum Ascomycota. It is

alkali tolerant yeast found in the sediment of Lake Vanda, Antarctica (Joseph, 2006). The two lipase variants from this organism viz., Lipase A and Lipase B have proven of particular interest to the researchers. Martinelle and Hult (1995) conducted the comparative studies on the interfacial activation of these lipases A and B with *Humicola lanuginose* lipase. The characterization of lipase A for substrate specificity and its utility as biocatalyst was reported (Kirk and Christensen, 2002). Further, the kinetics of acyl transfer reactions in organic media catalyzed by lipase B were studied (Martinelle and Hult, 1995). Anderson et al. (1998) determined the applications of lipase B in organic synthesis and the enantioselectivity of lipase for some synthetic substrates. Rotticci et al. (1998) proposed the molecular recognition of alcohol enantiomers by lipase B. The use of lipase B for the preparation of optically active alcohols was also determined (Rotticci et al., 2001). Studies revealed that size as a parameter for solvent effects on lipase B enantioselectivity. The evaluation of lipase as catalyst in different reaction media for hydrolysis of tributyrin as reaction model has been reported (Salis et al., 2003). The amidase activity of lipase B and structural feature of the substrates were reported (Torres et al., 2006). The performance of lipase B in the enantioselectivity esterifiction of ketoprofen (Ong et al., 2006) and the improvement of enantioselectivity of lipase (fraction B) via adsorption on polyethylenimine-agarose (Torres et al., 2006) were studied recently. The structure and activity of lipase B of *C. antarctica* in ionic liquids (van Rantwijk et al., 2006) and the applications of lipase B in organic synthesis has been reported (Anderson et al., 1998). Shimada et al. (2001) attempted the ethyl esterification of docosahexaenoic acid (DHA) in an organic solvent-free system using *C. antarctica* lipase, which acts strongly on DHA and ethanol. About 88% esterification was attained by shaking the mixture of DHA / ethanol (1:1, mol/mol) and 2 %wt immobilized *C. antarctica* lipase B at 30°C for 24 h. Use of lipase B from *C. antarctica* for the preparation of optically active alcohols has been reported (Rotticci et al., 2001).

Lipase produced by a psychrotroph, *P. fluorescens* P38, was found to catalyze the synthesis of butyl caprylate in n-heptane at low temperatures. The optimum yield of ester synthesis was 75% at 20°C with an organic phase water concentration of 0.25% (v/v). The results are discussed in terms of the structural flexibility of psychrotroph-derived lipase and the activity of this enzyme within a nearly anhydrous organic solvent phase (Tan et al., 1996).

Watanabe et al. (2002) found that the crude soybean oil did not undergo methanolysis with immobilized *C. antarctica* lipase but degummed oil did. The substance that was removed in the degumming step was estimated to inhibit the methanolysis of soybean triacylglycerols (TAGs). Methanolysis is the displacement of alcohol from an ester by methanol in a process similar to hydrolysis, except that methanol is employed instead of water. Methanol is widely used because of its low cost and its physical and chemical advantages. The main components of soybean gum are phospholipids (PLs), and soybean PLs actually inhibited the methanolysis reaction. Indeed, three-step methanolysis successfully converted 93.8% degummed soybean oil to its corresponding methyl esters, and could be reused for 25 cycles without any loss of the activity. This process widely reduced viscosity of triglycerides, thereby enhancing the physical properties the lipase of renewable fuels to improve engine performance. Lipase from *C. antarctica* has been evaluated as catalyst in different reaction media for hydrolysis of tributyrin as reaction model (Salis et al., 2003).

Applications in food Industry

In the food industry, reaction needs to be carried out at low temperature in order to avoid changes in food ingredients caused by undesirable side-reaction that would otherwise occur at higher temperatures. Lipases have become an integral part of the modern food industry. The use of enzymes to improve the traditional chemical processes of food manufacture has been developed in the past few years. Stead (1986) and Coenen et al. (1997) stated that, though microbial lipases are best utilized for food processing, a few, especially psychrotrophic bacteria of *Pseudomonas sp*. and a few moulds of *Rhizopus* sp. and *Mucor* sp. caused havoc with milk and dairy products and soft fruits. Cold active lipase from *Pseudomonas* strain P38 is widely used in non-aqueous biotransformation for the synthesis of n-heptane of the flavoring compound butyl caprylate (Tan et al., 1996). Immobilized lipases from *C. antarctica* (CAL-B), *C. cylindracea AY*30, *H. lanuginosa*, *Pseudomonas* sp. and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil Buisman et al. (1998).

Domestic applications

The most commercially important field of application for hydrolytic lipases is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. Godfrey and West (1996) reported that about 1000 t of lipases are sold every year in the area of detergents. The use of cold active lipase in the formulation of detergents would be of great advantage for cold washing that would reduce the energy consumption and wear and tear of textile fibers (Feller and Gerday, 2003). The industrial dehairing of hides and skin at low temperature using psychrophilic protease or keratinase would not only save energy but also reduce the impacts of toxic chemicals used in dehairing.

Enzymes can reduce the environmental load of detergent products since they save energy by enabling a lower wash temperature to be used; allow the content of other

often less desirable chemicals in detergents. Addition of cold active lipsase in detergent become biodegradable, leaving no harmful residues, have no negative impact on sewage treatment processes and do not present a risk to aquatic life. Commercial preparations used for the desizing of denim and other cotton fabrics contain both alpha amylase and lipase enzymes. Lipases are stable in detergents containing protease and activated bleach systems. Lipase is an enzyme, which decomposes fatty stains into more hydrophilic substances that are easier to remove than similar non-hydrolyzed stains (Fuji et al., 1986). The low temperature active lipase can be added to detergents to hydrolyze oily stains at the temperature of tap water to reduce energy consumption and protect the color of fabrics (Feller and Gerday, 2003).

The other common commercial applications for detergents is in dish washing, clearing of drains clogged by lipids in food processing or domestic/industrial effluent treatment plants (Bailey and Ollis, 1986). The use of cold active lipase as a liquid leather cleaner (Kobayashi, 1989) and as an ingredient in bleaching composition (Nakamura and Nasu, 1990) has been reported. Similarly its use in decomposition of lipid contaminants in dry-cleaning solvents (Abo, 1990), contact lens cleaning (Bhatia, 1990), degradation of organic wastes on the surface of exhaust pipes, toilet bowls, etc. (Moriguchi et al., 1990) have been reported. The removal of dirt/cattle manure from domestic animals by lipases and cellulases (Abo, 1990), washing, degreasing and water reconditioning by using lipases along with oxidoreductases, which allows for smaller amounts of surfactants and operation at low temperatures (Novak et al., 1990) have been proposed. The lipase component causes an increase in detergency and prevents scaling. The cleaning power of detergents seems to have peaked; all detergents contain similar ingredients and are based on similar detergency mechanisms. To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes, such as protease, amylase, cellulase and lipase (Ito et al., 1998).

Environmental applications

There are number of uses of the cold active enzymes, presently it is conceivable that they could be used for environmental bioremediation e.g., as a biodegradable means of treating an oil spill such as that which occurred by the Exon Valdese in Arctic water. Bioremediation for waste disposal is a new avenue in lipase biotechnology. Cheng et al. (1997) characterized cold-adapted organophosphorus acid anhydrolases for application in the efficient detoxification of pesticide and nerve agents. According to Buchon et al. (2000), cold adapted lipases have great potential in the field of wastewater treatment, bioremediation in fat contaminated cold environment, active compounds synthesis in cold condition. Further, more efforts are needed in identifying and cloning of novel lipase genes for environmental applications. Suzuki et al. (2001) identified a psychrotrophic strain of the genus *Acinetobacter* strain No. 6, producing an extracellular lipolytic enzyme that efficiently hydrolyzed triglycerides, such as soybean oil during bacterial growth even at 4°C. The strain degraded 60% of added soybean oil (initial concentration, 1% w/v) after cultivation in LB medium at 4°C for 7 days. The psychrophilic microorganisms as well as their enzymes have been proposed as alternative to physicochemical methods for bioremediation of solids and waste waters polluted by hydrocarbons, oils and lipids (Margesin et al., 2002). Belousova and Shkidchenko (2004) isolated 30 strains including *Pseudomonas* sp. and *Rhodococcus* sp. capable of oil degradation at 4-6°C and maximum degradation of masut and ethanol benzene resins were observed in *Pseudomonas* sp. and maximum degradation of petroleum oils and benzene resins were observed in *Rhodococcus* sp. Further, they stated that the introduction of psychrotrophic microbial degraders of oil products into the environment is most important in the contest of environmental problems in temperate regions. Ramteke et al. (2005) stated that in temperate regions, large seasonal variations in temperature reduce the efficiency of microorganisms in degrading pollutants such as oil and lipids. The lipase active at low and moderate temperature may also be ideal for bioremediation process.

Future outlook

In spite of the importance of cold active lipases, studies on the mechanisms of production of microbial lipases and the role of lipidic substances used as inducers in lipase production are scanty. Cold active lipases represent an extremely versatile group of bacterial extracellular enzymes that are capable of performing a variety of important reactions, thereby presenting a fascinating field for future research. The understanding of structure-function relationships will enable researchers to tailor new lipases active at low temperatures for biotechnological applications. Developments in research are expected from interchange of experiences between biochemists, geneticists and biochemical engineers. Wide and constant screening of new microorganisms for their lipolytic enzymes at low temperature will open novel and simpler routes for the synthetic processes. Consequently, this may pave new ways to solve biotechnological and environmental problems.

REFERENCES

Abdou AM (2003). Purification and Partial Characterization of Psychrotrophic *Serratia marcescens* Lipase. J. Dairy Sci. 86:127–132.

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

Alford JA, Pierce DA (1961). Lipolytic activity of microorganisms at low and intermediate temperatures. Activity of microbial lipases at temperatures below 0°C. J. Food Sci. 26:518-524.

Alquati C, De Gioia L, Santarossa G, Alberghina L, Fantucci P, Lotti M (2002). The cold-active lipase of *Pseudomonas fragi*: Heterologous expression, biochemical characterization and molecular modeling. Eur. J. Biochem. 269: 3321-3328.

Anderson EM, Larsson KM, Kirk O (1998). One biocatalyst – many applications: the use of *Candida antarctica* B-lipase in organic synthesis. Biocatalysis Biotransform. 16:181-204.

Andersson RE (1980). Microbial lipolysis at low temperatures. Appl. Environ. Microbiol. 39: 36-40.

Aoyama S, Yoshida N, Inouye S (1988). Cloning, sequencing and expression of the lipase gene from *Pseudomonas fragi* IFO-12049 in *E. coli*. FEBS Lett. 242: 36–40.

Arpigny JL, Lamotte J, Gerday C (1997). Molecular adaptation to cold of an Antarctic bacterial lipase. J. Mol. Catal. B Enzy. 3: 29-35.

Bailey JE, Ollis DF (1986). Applied enzyme catalysis. In: Biochemical Engineering fundamentals. 2nd edn. Mc Graw-Hill, New York, pp. 157–227.

NI, Shkidchenko AN (2004). Low temperature degradation of oil products differing in the extent of condensation. Appl. Biochem. Microbiol. 40: 262-265.

Benjamin S, Pandey A (1998). *Candida rugosa* lipases: Molecular biology and Versatility in Biotechnology. Yeast. 14: 1069-1087.

Bhatia RP (1990). Contact lens cleaning composition containing an enzyme and a carboxylvinyl polymer. United States Patent 4,921,630,

Botta M, Cernia E, Corelli F, Manetti F, Soro S (1997). Probing the substrate specificity for lipases II. Kinetic and modeling studies on the molecular recognition of 2-arylpropionic esters by *Candida rugosa* and *Rhizomucor miehei* lipases. Biochem. Biophys. Acta. 1337: 302–310.

Bousquet MP, Willemot RM, Monsan P, Boures E (1999). Lipase catalyzed α-butylglucoside lactate synthesis in organic solvent for dermo-cosmetic application. J. Biotechnol. 68: 61.

Breuil C, Kushner DJ (1975). Lipase and esterase formation by psychrophilic and mesophilic *Acinetobacter* species. Can. J. Microbiol. 21: 423–433

Buchon L, Laurent P, Gounot AM, Guespin MJF (2000). Temperature dependence of extrcellular enzyme production by Psychotrophic and Psychrophilic bacteria. Biotechnol. Lett. 22: 1577-1581.

Buisman GJH, Helteren CTW, Kramer GFH, Veldsink JW, Derksen JTP, Cuperus FP (1998). Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol. Lett. 20: 131-136.

Cavicchioli R, Saunders KS, Andrews D, Sowers KR (2002). Low-temperature extremophiles and their applications. Curr. Opin. Biotechnol. 13: 253-261.

Cavicchioli R, Siddiqui KS (2004). Cold adapted enzymes. In: A Pandey, C Webb, CR Soccol, C Larroche (eds) Enzyme Technology, Asiatech Publishers, India, pp. 615-638.

Chang H, Liao H, Lee C, Shieh C (2004). Optimized synthesis of lipase-catalyzed biodiesel by Novozym 435. J. Chem. Tech. Biotech. 80: 307 – 312.

Cheng TC, Liu L, Wang B, Wu J, De Frank JJ, Anderson DM, Rastogi VK, Hamilton AB (1997). Nucleotide sequence of a gene encoding an organophosphorous nerve agent degrading enzyme from *Alter-amonas haloplanktis*. J. Ind. Microbiol. Biotechnol. 18: 49-55

Choo DW, Kurihara T, Suzuki T, Soda K, Esaki N (1998). A cold-adapted lipase on an Alaskan psychrotroph, *Pseudomonas* sp. strain B11-1: Gene Cloning and Enzyme Purification and Characterization. Appl. Environ. Microbiol. 64: 486-491.

Coenen TMM, Aughton P, Verhagan H (1997). Safety evaluation of lipase derived from *Rhizopus oryzae*: Summary of toxicological data. Food Chem. Toxicol. 35: 315–22.

Collins T, Meuwis MA, Stals I, Claeyssens M, Feller G, Gerday C (2002).. A novel Family 8 Xylanase. Functional and Physicochemical Characterization. J. Biol. Chem. 277: 35133-35139.

Cui Y, Wei D, Yu J (1999). Lipase-catalyzed esterification in organic solvent to resolve racemic naproxen. Biotechnol. Lett. 19: 865-868.

Delille D (1993). Seasonal changes in the abundance and composition of marine heterotrophic bacterial communities in an Antarctic coastal area. Polar Biol. 12: 205-210.

Dieckelmann M, Johnson LA, Beacham IR (1998). The diversity of lipases from psychrotrophic strains of *Pseudomonas:* A Novel Lipase from a highly lipolytic strain of *Pseudomonas fluorescens*. J. Appl. Microbiol. 85: 527–536.

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

Feller G, Narinx E, Arpigny JL, Aittaleb M, Baise E, Geniot S, Gerday C (1996). Enzymes from Psychrophilic Organisms, FEMS Microbiol. Rev. 18: 189 -202.

Feller G, Thiry M, Arpigny JL, Gerday C (1991). Cloning and Expression in *Escherichia coli* of Three Lipase encoding genes from the Psychrotrophic Antarctic strain *Moraxella* TA144. Gene. 102:111–115.

Feller G, Thiry M, Arpigny JL, Mergeay M, Gerday C (1990). Lipases from Psychrotrophic Antarctic bacteria. FEMS Microbiol. Lett. 66: 239–244.

Fuji T, Tatara T, Minagwa M (1986). Studies on Application of lipolytic enzyme in Detergency I. J. Am. Oil Chem. Soc. 63: 796-799.

Ganapati DY, Piyush SL (2005). Lipase catalyzed transesterification of methyl acetoacetate with n-butanol J. Mol. Cat. B Enzy. 32: 107-113.

Georlette D, Blaise V, Collins T, Amico SD, Gratia E, Hoyoux A, Marx JC, Sonan G, Feller G, Gerday C (2004). Some like it cold: biocatalysis at low temperatures, FEMS Microbiol. Rev. 28: 25-42.

Gerday C, Aittaleb M, Arpigny JL, Baise E, Chessa JP, Garssoux G, Petrescu I, Feller G (1997). Psychtrophilic Enzymes: a Thermody-namic Challenge. Biochem. Biophys. Acta. 1342: 119-131.

Gerday C, Aittaleb M, Bentahir M, Chessa JP, Claverie P, Collins T, D'Amico S, Dumont J, Garsoux G, Georlette D, Hoyoux A, Lonhienne T, Meuwis M, Feller G (2000). Cold adapted enzymes: from fundamentals to biotechnology. Trend. Biotechnol. 18: 103-107.

Giudice AL, Michaud L, de Pascale D, Domenico MD, di Prisco G, Fani R, Bruni V (2006). Lipolytic activity of Antarctic Cold adapted marine bacteria. J. Appl. Microbiol. 101: 1039-1048.

Godfrey T, West S (1996). The application of enzymes in industry. In: T Godfrey, J Reichelt (eds) Industrial enzymology, 2nd (edn). The Nature Press, New York, p. 512.

Gomes J, Steiner W (2004). The Biocatalytic Potential of Extremophiles and Extremozymes. Food Technol. Biotechnol. 42: 223-235.

Ito S, Kobayashi T, Ara K, Ozaki K, Kawai S, Hatada Y (1998). Alkaline detergent enzymes from Alkalophiles: Enzymatic Properties, Genetics and Structures. Extremophiles. 2: 185 -190.

Jaeger KE, Eggert T (2002)**.** Lipases for biotechnology. Curr. Opin. Biotechnol. 13(4): 390–397.

Joseph B (2006) Isolation, purification and characterization of cold adapted extracellular lipases from psychrotrophic bacteria: Feasibility as laundry detergent additive. Ph.D Thesis. Allahabad Agricultural Institute – Deemed University, Allahabad, India.

Joseph B, Ramteke PW, Kumar PA (2006). Studies on the enhanced production of extracellular lipase by *Staphylococcus epidermidis.* J. Gen. Appl. Microbiol. 52: 315-320.

Khan IM, Dill CW, Chandan RC, Shahani KM (1967). Production and properties of the extracellular lipase of *Achromobacter lipolyticum*. Biochem.Biophys. Acta. 132: 68-77.

Kirk O, Christensen MW (2002). Lipases from *Candida antarctica*: Unique biocatalysts from a unique origin. Organic Process Research and Development. 6: 446-451.

Kobayashi H (1989). Liquid leather cleaners, Japanese Patent 1: 225-700.

Koops BC, Papadimou E, Verheij HM, Slotboom AJ, Egmond MR (1999). Activity and stability of chemically modified *Candida antarctica* lipase B absorbed on solid supports. Appl. Microbiol. Biotechnol. 52:791-796

Kulakovaa L, Galkina A, Nakayamab T, Nishinob T, Esakia N (2004). Cold-active esterase from *Psychrobacter* sp. Ant300: Gene cloning, Characterization, and the Effects of Gly Pro substitution near the active site on its catalytic activity and stability. Biochemica. Biophysica. Acta. 1696: 59– 65

Lee HK, Min JA, Sung HK, Won HS, Byeong CJ (2003). Purification and Characterization of Cold Active Lipase from Psychrotrophic *Aeromonas* sp. LPB 4. J. Microbiol. 41: 22-27.

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

Margesin R, Schinner F (1999). Cold-Adapted Organisms—Ecology,

Physiology, Enzymology and Molecular Biology. Springer-Verlag, Berlin, p. 416.
Martinelle M, Hult K (1995). Kinetics of acyl transfer reactions in organic media catalyzed by *Candida antarctica* lipase B. Biochemical Biophysica. Acta. 1251: 191-197.
Mayordomo I, Randez Gil F, Prieto JA (2000). Isolation, Purification and Characterization of a cold-active lipase from *Aspergillus nidulans*. J. Agric. Food Chem. 48: 105-109.
Moriguchi H, Hirata J, Watanabe T (1990). Microorganism based agent for treatment of organic wastes, Japanese Patent 2: 105,899.
Nakamura K, Nasu T (1990). Enzyme containing bleaching composition, Japanese Patent 2: 208,400.
Narinx E, Baise E, Gerday C (1997). Subtilisin from Psychrophilic Antarctic bacteria: characterization and site directed mutagenesis of residues possible involved in the adaptation to cold. Protein Eng. 10: 1271-1279.
Novak J, Kralova B, Demnerova K, Prochazka K, Vodrazka Z, Tolman J, Rysova D, Smidrkal J, Lopata V (1990). Enzyme agent based on lipases and oxidoreductases for washing, degreasing and water reconditioning, European Patent 355,228.
Ohgiya S, Hoshino T, Okuyama H, Tanaka S, Ishizaki K (1999). Biotechnology of enzymes from cold adapted microorganisms. In: R Margesin, F Schinner (eds) Biotechnological applications of cold adapted organisms, Springer- Verlag, Berlin Heidelberg, pp 17- 34.
Ong AL, Kamaruddin AH, Bhatia SW, Long WS, Lim ST, Kumari R (2006). Performance of free *Candida antarctica* lipase B in the enantioselective esterification of (*R*)-ketoprofen. Enzym. Microb. Technol. 39: 924-929.
Otto Y, Sawamoto T, Hasuo M (2000). Tributyrin specifically induces a lipase with a preference for the *sn-2* position of triglyceride in *Geotrichum* sp. F0401B, Biosci. Biotechnol. Biochem. 64: 2497-2499.
Patkar SA, Bjorking F, Zundel M, Schulein M, Svendsen A, Heldt Hansen HP, Gonnsen E (1993). Purification of two lipases from *Candida antarctica* and their inhibition by various inhibitors. Ind. J. Chem. 32: 76-80.
Patkar SA, Svendsen A, Kirk O, Groth IG, Borch K (1997). Effect of mutation in non-consensus Thr-X-Ser-X-Gly of *Candida antarctica* lipase B on lipase specificity, specific activity and thermostabilty. J. Mol. Catal. B Enzym. 3: 51–54.
Pemberton JM, Stephen PK, Radomir S (1997). Secreted enzymes of *Aeromonas*. FEMS Microbiol. Lett. 152:1-10.
Pirozzi D, Greco GJ (2004). Activity and stability of lipases in the synthesis of butyl lactate. Enzym. Microb. Technol. 34: 94-100.
Preuss J, Kaiser I, Gehring U (2001). Molecular characterization of a phosphatidylcholine - hydrolyzing phospholipase C. Eur. J. Biochem. 268: 5081-5091.
Ramteke PW, Joseph B, Kuddus M (2005). Extracellular lipases from anaerobic microorganisms of Antarctic. Ind. J. Biotech. 4:293-294.
Rashid N, Yuji S, Satoshi E, Haruyuki A, Tadayuki I (2001). Low-temperature lipase from psychrotrophic *Pseudomonas* sp. Strain KB700A. Appl. Environ. Microbiol. 67: 4064 - 4069.
Rotticci D, Haffner F, Orrenius C, Norin T, Hult K (1998). Molecular recognition of sec-alcohol enantiomers by *Candida antarctica* lipase B. J. Mol. Catal. B Enzy. 5: 267-272.
Rotticci D, Ottosson J, Norin T, Hult K (2001). *Candida antarctica* lipase B: A tool for the preparation of optically active alcohols. Methods in Biotechnol. 15: 261-276.
Russell RJM, Gerike U, Danson MJ, Hough DW, Tayalor GL (1998). Structural Adaptations of the cold active citrate synthase from an Antarctic Bacterium, Struture. 6: 351–361.
Ryu HS, Kim HK, Choi WC, Kim MH, Park SY, Han NS, Oh TK, Lee JK (2006). New cold-adapted lipase from *Photobacterium lipolyticum* sp. nov. that is closely related to filamentous fungal lipases. Appl. Microbiol. Biotechnol. 70: 321–326.
Salis A, Svensson I, Monduzzi M, Solinas V, Adlercreutz P (2003). The atypical lipase B from *Candida antarctica* is better adapted for organic media than the typical lipase from *Thermomyces lanuginosa*. Biochem. Biophys. Acta. 1646: 145-151.
Santarossa G, Lafranconi PG, Claudia A, Luca DG, Lilia A, Piercarlo F, Lotti M (2005). Mutations in the ''lid'' region affect chain length specificity and thermostability of a *Pseudomonas fragi* lipase. FEBS Lett. 579: 2383-2386.
Saxena RK, Sheoran A, Giri B, Davidson WS (2003). Purification strategies for microbial lipases. J. Microbiol. Meth. 52: 1–18.
Sharma R, Chisti Y, Banerjee UC (2001). Production, purification, characterization and applications of lipases. Biotechnol. Adv. 19: 627-662.
Shimada Y, Watanabe Y, Sugihara A, Baba T, Ooguri T, Moriyama S, Terai T, Tominaga Y (2001). Ethyl esterification of docosahexaenoic acid in an organic solvent-free system with immobilized *Candida antarctica* lipase. J. Biosci. Bioeng. 92: 19-23.
Siddiqui KS, Cavicchioli R (2005). Improved thermal stability and activity in the cold-adapted lipase B from *Candida antarctica* following chemical modification with oxidized polysaccharides. Extremophiles. 9: 471–476.
Sih CJ, Wu SH (1989). Resolution of enantiomers via biocatalysts. Topics Stereochem. 19: 63–125.
Slotema WF, Sandoval G, Guieysse D, Straathof AJJ, Marty A (2003). Economically pertinent continuous amide formation by direct lipase-catalyzed amidation with ammonia. Biotechnol. Bioeng. 82: 664 – 669.
Smidt H, Fischer A, Fischer P, Schmid RD (1996). Preparation of optically pure chiral amines by lipase catalyzed enantioselective hydrolysis of *N*-acyl-amines. Biotechnol. Tech. 10: 335-338.
Stead R (1986). Microbial Lipases their characteristics, role in food spoilage & industrial uses. J. Dairy Res. 53: 481-505.
Stinson SC (1995). Fine and intermediate chemical markers emphasize new products and process. Chem. Eng. News. 73: 10-26
Sullivan CW, Palmisano AC (1984). Sea ice microbial communities: Distribution, abundance and diversity of ice bacteria in Mc Murdo Soil, Antarctica, in 1980. Appl. Environ. Microbiol. 47:788-795.
Suzuki T, Nakayama T, Kurihara T, Nishino T, Esaki N (2001). Cold-active lipolytic activity of psychrotrophic *Acinetobacter* sp. strain no. 6. J. Biosci. Bioeng. 92: 144–148.
Tan S, Owusu ARK, Knapp J (1996). Low temperature organic phase biocatalysis using cold-adapted lipase from psychrotrophic *Pseudomonas* P38. Food Chem. 57: 415-418.
Torres R, Ortiz C, Pessela BCC, Palomo JM, Mateo C, Guisan JM, Fernandez LR (2006). Improvement of the enantioselectivity of lipase (fraction B) from *Candida antarctica* via adsorpiton on polyethylenimine-agarose under different experimental conditions. Enzym. Microb. Technol. 39: 167-171.
Uppenberg J, Hansen MT, Patkar S, Jones TA (1994b). The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida Antarctica*. Struct. 2 293-308.
Uppenberg J, Patkar S, Bergfors T, Jones TA (1994a). Crystallization and preliminary X-ray studies of lipase B from *Candida antarctica*. J. Mol. Biol. 235: 790-792.
van Rantwijk F, Secundo F, Sheldon RA (2006). Structure and activity of *Candida antarctica* lipase B in ionic liquids. Green Chem. 8: 282–286.
Vulfson EN (1994). Industrial applications of lipases. In: P Wooley, SB Petersen (eds) Lipases, Cambridge, Great Britain: Cambridge University Press. p. 271.
Watanabe Y, Shimada Y, Sugihara A, Tominaga Y (2002). Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antarctica* lipase. J. Mol. Catal. B Enzy. 17: 151-155.
Wintrode PL Miyazaki K, Arnold FH (2000). Cold adaptation of a mesophilic subtilisin-like protease by laboratory evolution. J. Biol. Chem. 275: 31635–31640.
Wiseman A (1995). Introduction to Principles. In: A Wiseman (ed) Handbook of enzyme biotechnology. 3rd ed. Padstow, Cornwall, UK: Ellis Horwood Ltd. T.J. Press Ltd., pp 3–8.
Yasufuku Y Ueji S (1996). Improvement five fold of enantioselectivtiy for lipase catalyzed esterification of a bulky substrate at 57 degree in organic solvent. Biotechnol. Tech. 10: 625-628
Yumoto I, Hirota K, Sogabe Y, Nodasaka Y, Yokota Y, Hoshino T (2003). *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea, Int. J. Syst. Evol. Microbiol. 53: 1985–1989.
Zeng X, Xiao X, Wang P, Wang F (2004). Screening and Characterization of psychrotrophic lipolytic bacteria from deep sea sediments. J. Microbiol. Biotechnol. 14: 952-958.
Zhang N, Suen WC, Windsor W, Xiao L, Madison V, Zaks A (2003). Im-

Improving tolerance of *Candida antarctica* lipase B towards irreversible thermal inactivation through directed evolution. Prot. Eng. 16: 599–605.

.