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Microbial Lipases and Their Industrial Applications: Review

[1]Berhanu Andualema and [2]Amare Gessesse
[1]Department of Biotechnology, Faculty of Natural and Computational Sciences,
University of Gondar, Ethiopia
[2]Biotechnology Program, Collage of Natural Sciences,
Addis Ababa University, Addis Ababa, Ethiopia

Abstract: Microbial lipases (triacylglycerol acyl-hydrolases, EC 3.1.1.3) catalyze both the hydrolysis and synthesis of long-chain acylglycerols. They are currently given much attention with the rapid development of enzyme technology. The chemo-, regio- and enantio-specific characteristics of lipase tends to be a focus research area for scientists and industrialists. Compared to plants and animals, microorganisms have been found to produce high yields of lipases. This review describes various industrial applications of microbial lipases in the area of food industry, oil and fat industry, detergent industry, pulp and paper industry, leather industry, textile industry, in organic synthesis, production of cosmetics and biodiesel production. This makes lipases the most widely used class of enzymes in different industrial activities through the application of bioprocess technology. The aim of this review is not to discuss every lipase described in the literature but rather to present recent information on the production, characterization and industrial application of lipases in our daily activities in order to improve our life styles.

Key words: Microbial lipases, esters, transesterification, industrial application

INTRODUCTION

Human beings have been using enzyme for different purposes starting from ancient civilizations. Today, nearly 4000 enzymes are known and of these, nearly 200 are in commercial use. At least 75% of all industrial enzymes (including lipases) are hydrolytic and the majority of the industrial enzymes are of microbial origin (Godfrey and West, 1996; Wilke, 1999).

Enzymes or microbial cells are used as biological catalysts due to their high specificity and economic advantages without any environmental impact. This is because; (1) Enzymes work better at gentle and available temperatures and other environmental conditions. It is possible to use enzymes instead of harsh conditions and harsh chemicals, as the result it could be helpful to save energy and prevent pollution. In the process, it does not use expensive corrosive-resistant equipment. (2) Enzymes are highly specific thus, the production of unwanted by products is avoided and there is no need to have extensive downstream processes. (3) Enzymes can be immobilized and can therefore be reuse several times. (4) Enzymes can also be used to treat waste consisting of harmful compounds. (5) Normally enzymes able to decompose naturally by help of decomposers, so the entire chemical components of the enzymes are readily recycled back to nature (Gennari *et al*., 1998). Furthermore, because of the many different bio-transformations that enzymes can catalyze, the numbers of enzymes used in commercial scale highly increased (Sharma *et al*., 2001a).

When we compare microbial enzymes with that of enzymes originated from plants or animals, microbial enzymes have variety of catalytic activities, high production capacity within a short period of time and ease for genetic manipulation. However, microbial enzymes can be produced at any time and do not affected by seasonal fluctuations. The other advantage of microorganisms over that of plants and animals is that they can grow rapidly on inexpensive media. Microbial enzymes are more stable interms of activity (Wiseman, 1995).

Only about 2% of the world's microorganisms have been tested as enzyme sources of which bacteria isolates take higher share than yeasts (Frost and Moss, 1987) and tend to have neutral or alkaline pH optima and are often thermostable. Currently, genetic and environmental

Corresponding Author: Berhanu Andualema, Department of Biotechnology, Faculty of Natural and Computational Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia Tel: +251918700027 Fax: 25158141931

manipulation techniques help to increase the yield of cells (Demain, 1971), through the conversion of inducible enzyme into constitutive type or it is also possible to increase the enzyme activity by inducing it or modifying the enzyme under utilization (Betz *et al*., 1974) using microbial cells. This is because microorganisms have short generation times, relatively simple nutritional requirements and since screening procedures for the desired characteristic are comparatively easier than higher organisms.

Enzymes are widely applied in the field of scientific research, cosmetic production, medical diagnostics and chemical analyses, therapeutic applications and industrial catalysis in the special syntheses (Sharma *et al*., 2001b). Of those well known significant enzymes, lipases have unique characteristics that they can carry out reactions at the interface between aqueous and non-aqueous media. This is primarily due to their ability to utilize relatively a wide spectrum of substrates, high stability towards extremes of temperature, pH and organic solvents, do not require cofactors. The enantioselective and regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesis of coca butter constituents, biofuels and for synthesis of personal care products and flavour enhancers (Jaeger and Reetz, 1998). Therefore, the chemo-, regio- and enantio-specific characteristics of these enzymes has caused tremendous interest among scientists and industrialists (Saxena *et al*., 2003).

Based on three-dimensional structure of various lipases, all have been classified as serine hydrolases (Winkler *et al*., 1990; Jaeger *et al*., 1993). This is because of the active site that composed of the catalytic triad Ser-Asp (Glu)-His is similar to serine proteases (Derewenda and Sharp, 1993; Brumlik and Buckley, 1996). However, the natural substrates of lipases are triglycerides having very low solubility in water. Under normal conditions, lipases catalyze the hydrolysis of ester bonds of triglycerides to glycerol and free fatty acids at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. That means, in contrast to esterases, lipases are activated only when adsorbed to an oil-water interface (Martinelle *et al*., 1995) and could not hydrolyze dissolved substrates in the bulk fluid. In contrast, esterases show normal Michaelis-Menten kinetics in aqueous solution. A true lipase will split emulsified esters of glycerine and long-chain fatty acids (Fig. 1). As it has been investigated at experimental conditions, such as, they are capable of reversing the reaction in the absence of water. This reverse reaction form glycerides from fatty acids and glycerol through esterification reaction. Lipases are also used to catalyze the transesterification reaction using alcohol together with fats, oils and free fatty acids to produce alkyl esters (biodiesel). The use of lipases to catalyze the transesterification of fatty acids to alkyl esters for use as biodiesel has not yet been commercially applied. However, the process has been investigated experimentally (Fig. 2).

Fig. 1: The catalytic action of lipases: A triglyceride can be hydrolyzed to form glycerol and fatty acids, or the reverse (synthesis) reaction can combine glycerol and fatty acids to form the triglyceride

Fig. 2: Lipase mediated alcoholytic transesterification of fats or oils and free fatty acids Freedman *et al*. (1986)

Because of their wide-ranging significance, lipases remain a subject of intensive study (Bornscheuer, 2000). Research on lipases is focused particularly on structural characterization, general characterization of performance (Bornscheuer, 2000) and industrial applications. In comparison with this effort, relatively little work has been done on development of lipase bioreactor systems for commercial use.

Development of lipase-based technologies for the synthesis of novel compounds is rapidly expanding the uses of these enzymes (Liese *et al*., 2000). An increasing number of lipases with suitable properties are becoming available and efforts are underway to commercialize biotransformation and synthesis based on lipases (Liese *et al*., 2000).

In conclusion, lipases catalyze both the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids (Fig. 1). These reactions usually proceed with high region- and /or enantioselectivity, making lipases an important group of biocatalysts in organic chemistry. The reasons for the enormous biotechnological potential of microbial lipases include the facts that they are (1) stable in organic solvents, (2) do not require cofactors, (3) possess broad substrate specificity (4) act over a wide range of pH and temperature and (5) exhibit a high enantioselectivity. Currently, lipases are produced by animals, plants and microorganisms. The most commonly animal lipase is produced from pancreatic gland. With regard to plant, papaya latex, oat seed land castor seed can serves source of lipase (Akoh *et al*., 2007). Microorganisms have been found to produce high yields of lipases compare to the animal and plants. This is because their production, commercialization and application at industrial scale is more simple than animal and plant ones (Akoh *et al*., 2007; Antczak *et al*., 2009). Lipases produced from microorganisms such as bacterial and fungal are widely applied in the field of biotechnology and organic chemistry. As the result, most commercially produced lipases have been produced from fungi and bacteria. Among lipase producing organisms, Table 1 lists those microbes that appear to be the most widely used in biotechnology (Jaeger and Reetz, 1998).

The high-level production of microbial lipases requires not only the efficient over expression of the corresponding genes but also a detailed understanding of the molecular mechanisms governing their folding and secretion. The optimization of industrially relevant lipase properties can be achieved by directed evolution. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantio-pure pharmaceuticals, agrochemicals and flavour compounds (Jaeger and Eggert, 2002). 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 (Vulfson, 1994). The chemo-, regio- and enantiospecific behavior of these enzymes has caused tremendous interest among scientists and industrialists (Saxena *et al*., 2003).

Lipases from a large number of bacterial, fungal and plant and animal sources have been purified to homogeneity (Saxena *et al*., 2003). Lipases isolated from different sources have a wide range of properties depending on their sources with respect to positional specificity, fatty acid specificity, thermostability, pH optimum, etc. (Huang, 1984). The objective of this review is not to discuss every lipase and lipase related points in the literature but rather to present information on selected concepts of lipases and their applications at industrial scale. Therefore, the paper reviews the fundamental knowledge available on lipases, with particular emphasis on isolation, production, purification, three-dimensional structure, immobilization and thermostability of lipases. Moreover, it focuses on the industrial applications of lipases in the area of food industry, oil and fat industry, detergent industry, pulp and paper industry, leather industry, textile industry, in organic synthesis, production of cosmetics and biodiesel production.

WHAT EXACTLY IS A LIPASE?

Currently, there is no inclusive answer to this simple statement question. Until recently, two criteria have been used to classify a lipolytic enzyme as a “true” lipase (EC 3.1.1.3): (a) it should be activated by the presence of an interface, that is, its activity should sharply increase as soon as the triglyceride substrate forms an emulsion. This is commonly known as interfacial activation (Sarda and Desnuelle, 1958). (b) It should contain a “lid” which is a surface loop of hydrophobic oligo-peptide covering the active site of the enzyme and moving away on contact with the interface and immediately the substrate enter the binding pocket (Van Tilbeurgh *et al*., 1993). However, these obviously suggestive criteria are not suitable for classification, mainly because of the presence of a number of exceptions. Some lipases have a lid but not exhibiting interfacial activation (Verger 1997). Even there are microbial lipases lacking a lid that covers the active site in the absence of lipid-water interfaces. Therefore, lipases are simply defined as carboxyl-esterases catalyzing the hydrolysis (and synthesis) of long-chain triglycerides (Ferrato *et al*., 1997). However, there is no strict definition available for the term “long-chain,” but glycerol esters with an acyl chain length of greater than 10 carbon atoms can be regarded as lipase substrates, with triglyceride being the standard substrate. One lipase type differs from the others in length and architecture of the binding domain of the α/β-hydrolase fold proteins. That is why lipases have wide range of substrate diversity.

BASIC CHARACTERISTICS OF LIPASES

The lipases produced by organisms can be widely used for different activities in different form, such as extracellular, intracellular, immobilized and regiospecific. Extracellular lipase refers to the use of the enzyme that has been previously extracted from the producing organism and purified using different techniques. On the other

hand, intracellular lipase refers to the use of the enzyme while it is still contained in the producing organism (Robles-Medina *et al*., 2009). Both extracellular and intracellular lipase could be immobilized using a solid support (Jegannathan *et al*., 2008). They can also be regiospecific by nature which means they only act on specific bonds of the triglyceride molecule (Robles-Medina *et al*., 2009).

Extracellular lipase: Microbial lipases are mostly extracellular which can be produced by submerged fermentation or solid state fermentation. The fermentation process is usually followed by purification process in order to increase the degree of purity thereby improve the biocatalyst activity of the enzyme (Balaji and Ebenezer, 2008; Barberis *et al*., 2008). The important purification step for producing extracellular lipase is a complex process and it depends on the origin and structure of the lipase (Saxena *et al*., 2003). The large scale production of extracellular lipases should be economical, fast, easy and efficient. Unfortunately, the cost of novel purification technologies is higher than production (Joseph *et al*., 2008). The majority of immobilized lipases which are commercially available are entirely extracellular (Robles-Medina *et al*., 2009). The most commonly used immobilized lipases are: Novozym 435, Lipozyme RM IM and Lipozyme TL IM which are secreted from *Candida antarctica*, *Rhizomucor miehei* and *Thermomyces lanuginosus*, respectively (Robles-Medina *et al*., 2009).

Intracellular lipase: the cost for purification of extracellular lipases is high and the alternative method used to solve this problem is the use of the whole cells as biocatalysts. Rather than using enzyme lipase, the use of compact cells as it is for intracellular production of lipases or fungal cells immobilized within porous biomass support particles as a whole biocatalyst represents an attractive process for bulk production of biodiesel and polyesters (Iftikhar *et al*., 2008). The utilization of lipase found in the cells is referred to as intracellular lipase (Robles-Medina *et al*., 2009). Some microorganisms used as source of lipase are able to be spontaneously immobilized on certain supports. This reduced or eliminates the costly purification step and the need for an extended immobilization process which is necessary in comparison with the extracellular lipase (Fukuda *et al*., 2001).

Isolation and screening of lipase-producing microorganisms: Lipases are produced by many microorganisms and higher eukaryotes. Most commercially useful lipases are of microbial origin. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds and decaying food (Sztajer *et al*., 1988), as well as from compost heaps, coal tips and hot springs (Wang *et al*., 1995). Some of the lipase-producing microorganisms are listed in Table 1.

Lipase-producing microorganisms belong to bacteria, fungi, yeasts and actinomyces. The lipases found among these microbial sources are quite diverse and typically vary from one another in physical, chemical and biological properties. Even although a large number of lipolytic enzymes are known in microorganisms, not all such enzymes are suitable for commercial utilization. Such factors as pH range, tolerance of emulsification and surfactants, temperature tolerance, storage capability and the like are important considerations in the selection and development of a commercially useful product (Sharma *et al*., 2001a).

A simple and reliable method for detecting lipase activity in microorganisms has been described by Sierra (1957). This method uses the surfactant Tween 80 in a solid medium to identify a lipolytic activity. The formation of opaque zones around the colonies is an indication of lipase producing organisms. Modifications up on this assay have been carried out, like the use of various Tween surfactants in combination with Nile blue or neatsfoot oil and Cu^{2+} salts. On the other hand, screening of lipase producers on agar plates is frequently done by using tributyrin as a substrate (Cardenas *et al*., 2001) and clear zones around the colonies indicate production of lipase. Screening systems making use of chromogenic substrates have also been described (Yeoh *et al*., 1986). The most widely used substrates are tributyrin and triolein which are emulsified mechanically in various growth media and poured into a plate. Lipase production is indicated by the formation of clear halos around the colonies grown on tributyrin-containing agar plates (Atlas, 1996) and orange-red fluorescence visible on irradiation with a conventional UV hand lamp at 350 nm on triolein plates with rhodamine B are widely used (Kouker and Jaeger, 1987).

Lipase activity in bacterial culture supernatants is determined by hydrolysis of p-nitrophenylesters of fatty acids with various chain lengths and spectrophotometric detection of p-nitrophenol at 410 nm. A more laborious but reliable method for identifying a "true" lipase is the determination of fatty acids liberated from a triglyceride by titration (Jensen, 1983).

Currently, lipases are not only used to hydrolysis of substrates but also important for synthesis of different substances from monomers. A standard reaction is the lipase-catalyzed esterification of an alcohol with a

Table 1: Lipases producing microorganisms

Species	References
Acinetobacter radioresistens	Chen *et al.* (1998)
Aeromonas hydrophila	Pemberton *et al.* (1997)
Aeromonas sobria	Lotrakul and Dharmsthiti (1997)
Aeromonas sp.	Lee *et al.* (2003)
Aspergillus oryzae	Chen *et al.* (1998)
Bacillus cereus	El-Shafei and Rezkallah (1997)
Bacillus coagulans	El-Shafei and Rezkallah (1997)
Bacillus stearothermophilus	Kim *et al.* (1998)
Bacillus sp.	Wang *et al.* (1995)
Candida antarctica	Alford and Pierce (1961), Sih and Wu (1989), Vulfson (1994), Buisman *et al.* (1998), Arroyo *et al.* (1999), Shimada *et al.* (1999) and Robles-Medina *et al.* (2009)
Candida rugosa	Jaeger and Reetz (1998)
Geotrichum candidum	Alford and Pierce (1961) and Buisman *et al.* (1998)
Geotrichum sp.	Lotrakul and Dharmsthiti (1997)
Humicola lanuginosa	Buisman *et al.* (1998) and Chen *et al.* (1998)
Penicillium roquefort	Alford and Pierce (1961)
Photobacterium lipolyticum	Ryu *et al.* (2006)
Pseudoalteromonas sp.	Zeng *et al.* (2004)
Pseudomonas aeruginosa	Sharon *et al.* (1998)
Pseudomonas alcaligenes	Chen *et al.* (1998)
Pseudomonas cepacia and	Kaieda *et al.* (2001)
Pseudomonas mendocina	Chen *et al.* (1998)
Pseudomonas sp.	Buisman *et al.* (1998), Rajmohan *et al.* (2002) and Feller and Gerday (2003)
Pseudomonas fluorescens	Kojima *et al.* (1994) and Rajmohan *et al.* (2002)
Psychrobacter sp.	Zeng *et al.* (2004)
Pyrococcus furiosus	Adams *et al.* (1995) and Fischer *et al.* (1996)
Thermotoga sp.	Adams *et al.* (1995) and Fischer *et al.* (1996)
Rhizomucor miehei	Pabai *et al.* (1995) and Robles-Medina *et al.* (2009)
Thermomyces lanuginosus	Robles-Medina *et al.* (2009) and Chen *et al.* (1998)
Yersinia enterocolitica	Glogauer *et al.* (2011)
Aeromonas sp.	Charoenpanicha *et al.* (2011)
Acinetobacter baylyi	Uttatree and Charoenpanich (2011) and Uttatree *et al.* (2010)
Burkholderia sp.	Yuan *et al.* (2010)
Pseudomonas aeruginosa KM110	Mobarak-Qamsari *et al.* (2011)
Acinetobacter junii	Anbu *et al.* (2011)

carboxylic acid (Reetz and Jaeger, 1998). The first speed of ester formation could be determined by gas chromatography. Currently, there is no standard or single method used to determine the enantioselectivity of a lipase-catalyzed organic reaction. As a result of this, the enantioselectivity of product formation is determined either by gas chromatography or High Performance Liquid Chromatography (HPLC), with chirally modified columns.

Production and media development for lipase: Microbial lipases are produced mostly by submerged culture (Ito *et al.*, 2001), but solid state fermentation methods (Chisti, 1999a) can be used also. The Solid State Fermentation (SSF) is an interesting alternative for microbial enzyme production due to the possibility of using residues and by-products of agro-industries as nutrient sources and support for microorganism development. The use of by-products as substrates for lipase production, adds high value and low-cost substrates may reduce the final cost of the enzyme (Menoncin *et al.*, 2008, Rodriguez *et al.*, 2006).

Many studies have been carried out to define the optimal culture and nutritional requirements for lipase production by submerged culture. However, production of lipase through submerged fermentation needs, large space, complex media and also needs complex machinery, equipment and control systems. Moreover, submerged fermentation for production of lipase at large scale demands high energy demand, higher capital and recurring expenditure (Satyanarayana, 1994).

Immobilized cell culture has been applied in a few cases (Hemachander *et al.*, 2001). In several cases, immobilization of microbial cells producing lipases increase the extent of reaction and facilitate the downstream processing. This is because it avoids washout of the cells at dilution rates, it helps to increase cell concentration in the reactor and easy separation of cells from the product containing solution (Gunasekaran and Das, 2005).

Generally, lipase production is influenced by the type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature and dissolved oxygen concentration (Elibol and Ozer, 2001). Lipidic carbon sources seem to be generally essential for obtaining a high lipase yield; however, a few authors have produced good yields in the absence of fats and oils (Shimada *et al.*, 1992).

Purification of lipases: Purification of lipase is essential for industries of fine chemicals, pharmaceuticals and cosmetics. It is also significant to investigate and understand the 3-D structure of the enzyme and their structure-function relationships (Saxena *et al.*, 2003). Currently many lipases have been widely purified and characterized without losing their activity and stability profiles depending to pH, temperature and effects of metal ions and chelating agents. The methods used for purification of lipases are nonspecific techniques, some of which are extraction, precipitation, hydrophobic interaction, chromatography, gel filtration, crystallization and ion exchange chromatography. Affinity chromatography is significant to reduce most purification steps needed (Woolley and Peterson, 1994). If the objective is production of lipase for industrial use, the purification technique should be inexpensive, rapid, high-yielding and liable to large-scale operations. The degrees of quality of the products are entirely to depend

on the purpose and the economic point of view. For instance, the lipase for synthetic reactions in pharmaceutical industry needs further purification (Koblitz and Pastore, 2006).

Shear tolerance of lipases: The application of strong mechanical agitation and emulsifiers can improve interfacial area in the bioreactors. However, a combination of interface and agitation sometimes affect the activity and stability of lipases. Extreme agitation and liquid-liquid interfaces are frequently common in lipase-assisted hydrolysis (Rooney and Weatherley, 2001). Shear-associated inactivation of many enzymes (Chisti, 1999b) including lipases (Lee and Choo, 1989; Mohanty *et al.*, 2001) at gas-liquid and liquid-liquid interfaces have been reported.

The rate of interfacial lipase denaturation is directly proportional to the increment of temperature (Lee and Choo, 1989) and turbulence in the fluid (Chisti, 1999b). That means, the denaturation rate constant depends on the specific power input in the reactor and the amount of gas-liquid interface present (Mohanty *et al.*, 2001). To avoid or reduce the rate of denaturation, addition of polypropylene glycol is significant (Lee and Choo, 1989). In this case, interfacial denaturation is mostly common by losing the 3-dimentional structure lipase rather than molecule breakage into multiple peptides (Lee and Choo, 1989).

Three-dimensional structure of lipases: The determination of the three-dimensional structures and the factors that determine their regiospecificity and enantiospecificity are essential to make suitable lipases for specific applications. From 1990 to 1998 the three dimensional structure of 12 types of lipases from different sources have been studied which, with the exception of pancreatic lipases, are all of microbial origin (Jaeger and Reetz, 1998 (Sharma *et al.*, 2001a). The molecular weights of these enzymes range from 19 to 60 kDa.

With regard to lipases structure, all had very similar folds despite a lack of amino acid sequence similarity (Cygler *et al.*, 1992; Smith *et al*, 1992). All have almost the same three-dimensional structure characterized as α/β-hydrolase folding (a specific sequence of α-helices and β-strands) (Balkenhohl *et al.*, 1997) with most of them containing a helical segment called the lid that covers the active site when the enzyme is in the so-called closed conformation. When lipid aggregates are available, the lid opens immediately and the enzyme activity is going to increased, a condition is termed as interfacial activation. Moreover, the lipase core is composed of a central β sheet that is composed of eight different β strands (β_1-β_8) connected by up to six α helices (A-F). Canonical fold of α/β-hydrolase is an ideal example for this case. The canonical α/β-hydrolase fold consists of a central, mostly parallel β sheet of eight strands with the second strand anti-parallel. The parallel strands range from β_3 to β_8 are connected by α helices which pack on either side of the central β sheet. The β sheet has a left-handed super-helical twist such that the surface of the sheet covers about half a cylinder and the first and last strands cross each other at an angle of 90^0. The curvature of the β sheet may differ significantly among the various enzymes and also, the spatial positions of topologically equivalent α helices may vary considerably. They differ substantially in length and architecture, in agreement with the large substrate diversity of these enzymes (Jaeger *et al*, 1999). The active site of the α/β-hydrolase fold enzymes composed of three catalytic residues which are referred as nucleophilic residue (serine, cysteine, or aspartate), a catalytic acid residue (aspartate or glutamate) and a histidine residue, always in this order in the amino acid sequence (Ollis *et al*, 1992). The order of the residues is different from that observed in any of the other proteins that contain catalytic triads. In lipases, the nucleophile has been found to be a serine residue, whereas the catalytic acid is either an aspartate or a glutamate residue. The nucleophilic Ser residue is located at the C-terminal end of strand β5 in a highly conserved pentapeptide GXSXG, forming a characteristic β-turn-α motif which is known as 'nucleophilic' elbow. The hydrolysis of the substrate is started with a nucleophilic attack by the catalytic-site-Ser oxygen on the carbonyl carbon atom of the ester bond, leading to the formation of a tetrahedral intermediate stabilized by hydrogen bonding to nitrogen atoms of main chain residues that belong to the so-called 'oxyanion hole'. An alcohol is produced and released from an acyl-lipase complex which is finally hydrolyzed with the production of the fatty acid and regeneration of the enzyme.

Immobilization of lipases: Enzyme immobilization increases the number of enzyme molecules per unit area increasing the efficiency of the reaction. Like with other enzymes, the advantages of immobilizing lipases include the repetitive use of a given batch of enzyme, better process control, enhanced stability, enzyme-free products (Rahman *et al.*, 2005), increased stability of polar substrates, shifting of thermodynamic equilibria to favour ester synthesis over hydrolysis, reduction of water dependent side reactions such as hydrolysis, elimination of microbial contamination and the potential for use directly within a chemical process. In the presence of

organic solvents, immobilized lipase has been showed enhanced activity (Ye *et al*., 2005).

Currently, many methods have been used to immobilize lipases, including adsorption or precipitation onto hydrophobic materials (Wisdom *et al*., 1984), covalent attachment to functional groups (Shaw *et al*., 1990), entrapment in polymer gels (Telefoncu *et al*., 1990), adsorption in macroporous anion exchange resins (Rizzi *et al*., 1992), microencapsulation in lipid vesicles (Balcao *et al*., 1996) and sol-gel entrapment (Krishnakant and Madamwar, 2001). Of those immobilized approaches, enzyme entrapment by help of inorganic matrixes such as silica gel makes more efficient (Shtelzer *et al*., 1992). In this regard, *Candida antarctica* B (Novozym 435) was immobilized on mesoporous silica with octyltriethoxysilane and it retained its activity even after 15 reaction cycles (Blanco *et al*., 2004). Calcium carbonate was found to be the most suitable adsorbent when crude *Rhizopus oryzae* lipase was immobilized on different supports and it exhibited long-chain fatty acid specificity (Ghamguia *et al*., 2004). The lipase from *Pseudomonas cepacia* was gel-entrapped by polycondensation of hydrolysed tetramethoxysilane and isobutyltrimethoxy silane and was subjected to repeated use without losing much of its activity (Noureddini *et al*., 2005).

Thermophilic and psychrophilic lipases of microbial origin: The optima activity of lipases obtained from conventional sources range from 30 and 60°C. However, currently, lipases were obtained from extremophiles, i.e., organisms adapted to life in high temperature, with maximum activity over 70°C (*Bacillus thermocatenulatus*) or with high activity at low temperature as is the case for enzymes produced by Antarctic bacteria, such as *Pseudomonas* and *Moraxella* sp. Such extreme and unusual features open the possibility to apply these enzymes without further modification using molecular engineering approaches to adapt them for use in reactions carried out at high temperatures or, conversely low temperature processes such as that of detergents (low temperature washes) or in food processing (Demiorijan *et al*., 2001). Generally, lipases are further divided into three based on their degree of temperature stability; namely psychrophilic, mesophilic and thermophilic. Thermostable enzymes can be obtained from mesophilic and thermophilic organisms; even psycrophiles have some thermostable enzymes (Adams *et al*., 1995). Currently, lipases from thermophilic and psychrophilic organisms have been proved to be more useful for biotechnological applications (Imamura and Kitaura, 2000). Therefore, this review only focuses on thermophilic and psychrophilic organisms.

Thermophilic lipases: The demand of thermostable lipases for different applications has been growing rapidly. Most of the studies were carried out to produce lipases from mesophilic microorganisms. Many lipases from mesophiles are stable at elevated temperatures (Sugihara *et al*., 1991). Proteins from thermophilic organisms have also been proved to be more useful for biotechnological applications than similar proteins from mesophiles due to their stability at high temperature (Imamura and Kitaura, 2000). Enzymes with high thermostability are important to have higher reaction rate at higher operation temperature. This is because higher temperature can increase solubility of substrates and also help to lower substrate viscosity and thereby avoid environmental contamination (Mozhaev, 1993).

Recently, biotechnologically significant enzymes were produced from hyperthermophilic archaebacteria, such as *Pyrococcus furiosus* and *Thermotoga* sp. (Adams *et al*., 1995) (Table 2). Thermostable lipases from such microbial sources are highly advantageous for biotechnological applications, since they can be produced at low cost and exhibit improved stability at high extreme temperature (Handelsman and Shoham, 1994). Currently, there has been a great demand for thermophilic and thermostable enzymes in various industrial fields. Thus, thermostable lipases from various sources have been purified and characterized using appropriate procedures (Sugihara *et al*., 1991). Lipases operating chemical reaction at elevated temperatures have the following advantages. (1) A higher diffusion rates. (2) Increased solubility of lipids and other hydrophobic substrates in water. (3) Decreased substrate viscosities. (4) Increased reactant solubility. (5) Higher temperature faster reaction rates. (6) Reduced risk of microbial contamination (Hasan *et al*., 2006) Thermophile microorganisms are a valuable source of thermostable lipase with desired properties usually associated with stability in solvents and detergents for potential biotechnological and industrial applications (Haki and Rakshit, 2003). These enzymes have been applied to synthesis biopolymers, pharmaceutical chemicals, agrochemicals, cosmetics, flavours and biodiesel (Haki and Rakshit, 2003).

Currently, thermostable lipases have been isolated from many sources, including *Pseudomonas fluorescens* (Kojima *et al*., 1994); *Bacillus* sp. (Wang *et al*., 1995); *B. coagulans* and *B. cereus* (El-Shafei and Rezkallah, 1997); *B. stearothermophilus* (Kim *et al*., 1997); *Geotrichum* sp. and *Aeromonas sobria* (Lotrakul and Dharmsthiti, 1997; Macedo *et al*., 1997) and *P. aeruginosa* (Sharon *et al*., 1998). The enzyme from *P. aeruginosa* was significantly stabilized by Ca^{2+} and was inactivated by EDTA. This inactivation could be overcome by adding $CaCl_2$, suggesting the existence of a calcium-binding site

Table 2: Thermophilic and Psychrophilic lipase producing microorganisms

Thermostable lipase producing microorganisms	References
P. fluorescens	Kojima *et al.* (1994)
Bacillus sp.	Wang *et al.* (1995)
B. coagulans	El-Shafei and Rezkallah (1997)
B. cereus	El-Shafei and Rezkallah (1997)
B. stearothermophilus	Kim *et al.* (1998)
Geotrichum sp.	Lotrakul and Dharmsthiti (1997)
A. sobria	Lotrakul and Dharmsthiti (1997)
P. aeruginosa	Sharon *et al.* (1998)
P. furiosus and *Thermotoga* sp.	Adams *et al.* (1995) and Fischer *et al.* (1996)
Psychrophilic lipase producing microorganisms	
A. hydrophila	Pemberton *et al.* (1997)
Aeromonas sp.	Lee *et al.* (2003)
Pseudoalteromonas sp.	Zeng *et al.* (2004)
Psychrobacter sp.	Zeng *et al.* (2004)
P. lipolyticum	Ryu *et al.* (2006)
C. antarctica	Buisman *et al.* (1998)
C. lipolytica	Alford and Pierce (1961)
G. candidum	Alford and Pierce (1961)
P. roqueforti	Alford and Pierce 1961)
Rhizopus sp. and *Mucor* sp.	Coenen *et al.* (1997)
Pseudomonas sp.	Feller and Gerday (2003)
Rhizopus sp. and *Mucor* sp.	Feller and Gerday (2003)

in *P. aeruginosa* lipase. One of the more notable thermostable enzymes was isolated by Wang *et al.* (1995) from a *Bacillus* strain. This enzyme had maximum activity at 60°C and retained 100% of the original activity after being held at 75°C for 30 min. The half-life of the enzyme was 8 h at 75°C (Wang *et al*., 1995). The enzyme retained at least 90% of the original activity after being incubated at 60°C for 15 h (Wang *et al*., 1995). Other highly thermostable lipases have been reported (Gao and Breuil, 1995; Kim *et al*., 1998; Lee *et al*., 1999).

Thermal stability of a lipase is clearly related with its structure (Zhu *et al*., 2001). Thermostability is also influenced by environmental factors such as pH and the presence of metal ions. At least in some instances, thermal denaturation appears to occur through intermediate states of unfolding of the polypeptide (Zhu *et al*., 2001). Mutations in the 'lid' region of the lipase can significantly affect heat stability (Zhu *et al*., 2001). Attempts are being made to protein engineer lipases for improved thermal stability. Compared to the native enzyme, thermal and operational stability of many lipases can be significantly enhanced by immobilization (Arroyo *et al*., 1999; Hiol *et al*., 2000). For instance, *C. antarctica* lipase B could be thermally stabilized by immobilization (Arroyo *et al*., 1999).

Psychrophilic lipases: Cold adapted lipases are largely distributed in microorganisms existing at low temperatures nearly 5°C. Although a number of lipase producing organisms 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. Various studies showed that a high bacteria count has been recorded as high as 10^5 and 10^6 per ml water column and in the sea ice, respectively (Delille, 1993). Cold adapted bacterial strains were isolated mostly from Antarctic and Polar regions which represent a permanently cold (0±2°C). A marine bacterium *Aeromonas hydrophila* growing at a temperature range between 4 and 37°C was found to produce cold active lipolytic enzyme (Pemberton *et al*., 1997). 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) and *Photobacterium lipolyticum* (Ryu *et al*., 2006). Permanently cold regions such as glaciers and mountain regions are another habitat for psychrophilic lipase producing microorganisms (Joseph *et al*., 2007). The soil and ice in Alpine region also harbor psychrophilic microorganisms which produces cold active lipases.

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 psychrophilic fungi. *Candida lipolytica, Geotrichum candidum* and *Penicillium roqueforti* have also been isolated from frozen food samples and reported to produce cold active lipases (Alford and Pierce, 1961). Psychrotrophic lipolytic moulds such as *Rhizopus* sp. and *Mucor* sp. were grown on milk and dairy products and soft fruits (Coenen *et al*., 1997).

Cold active lipases have lately attracted attention of communities 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.

With increasing interest in psychrophiles 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. Their current application 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 (Cavicchioli *et al*., 2002).

In summary, Enantioselective interesterification and transesterification have great significance in pharmaceutical for selective acylation and deacylation (Stinson, 1995). 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 (Vulfson, 1994). 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. 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 lipase together with protease or keratinase would not only save energy but also reduce the impacts of toxic chemicals used in dehairing. This is because they have no negative impact on sewage treatment processes and do not present a risk to aquatic life. The other common commercial application of lipase as detergent includes in dish washing, clearing of drains clogged by lipids in food processing or domestic/industrial effluent treatment plants (Bailey and Ollis, 1986). As determined by Buchon *et al*. (2000), cold adapted lipases have great potential in the field of wastewater treatment, bioremediation in fat contaminated cold environment and active compounds synthesis in cold condition.

INDUSTRIAL APPLICATIONS OF LIPASES

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of the versatility of their applied properties and ease of mass production. Microbial lipases are highly diversified in their enzymatic properties and substrate specificity which make them very attractive for industrial applications. Next to proteases and carbohydrases, lipases are considered to be the third largest group based on total sales volume. The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications (Jaeger *et al*., 1999). The majority of the enzymes used for industrial applications are of microbial origin and are produced in conventional aerobic submerged fermentations which allows greater control of the conditions of growth than solid-state fermentations (Cheetham, 1995). Currently lipases have received increased attention, evidenced by the increasing amount of information about lipases in the current literature. Lipases are valued biocatalysts because they act under mild conditions, are highly stable in organic solvents, show broad substrate specificity and usually show high regio- and/or stereoselectivity in catalysis (Snellman *et al*., 2002). The usefulness of bacterial lipase in commerce and research drives from its physiological and physical properties. A large amount of purified lipase could become available, i.e. ease of mass production. Bacterial lipases are generally more stable than animal or plant lipases. Lipases are active under ambient conditions and the energy expenditure required to conduct reactions at elevated temperatures and pressures is highly reduced and thereby the destruction of labile reactants and products are also highly reduced. Thermophilic microorganisms and enzymes stable at high temperatures and adverse chemical environments are of advantage in industrial uses. Due to high degree of specificity of enzymes, unwanted side products that normally appear in the waste stream are also reduced or eliminated. The use of enzymes can decrease the side reactions and downstream problems. One the unique characteristics of lipases is that they remain active in organic solvents in field of industrial application. When immobilized lipases are used under typical 'industrial' conditions, reactor temperatures as high 70°C are possible for prolonged periods.

Lipases are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacture and production of cosmetics (Rubin and Dennis, 1997). Lipase can be used to accelerate the degradation of fatty waste materials (Masse *et al*., 2001) and a synthetic plastic (polyurethane) (Takamoto *et al*., 2001). Some of industrial important microorganisms are presented on Table 3.

Lipases in food industry: Fats and oils are essential constituents of foods. The nutritional and sensory value

Table 3: Industrial applicable lipase produced from different microorganisms

Industrial lipase producing microorganisms	References
Pseudomonas sp.	Rajmohan *et al.* (2002)
P. fluorescens	Rajmohan *et al.* (2002)
C. antarctica	Buisman *et al.* (1998)
C. cylindracea	Buisman *et al.* (1998)
H. lanuginosa	Buisman *et al.* (1998)
Pseudomonas sp.	Buisman *et al.* (1998)
Geotrichum candidum	Buisman *et al.* (1998)
Rhizomucor miehei	Pabai *et al.* (1995)
Humicola lanuginosa	Chen *et al.* (1998)
Aspergillus oryzae	Chen *et al.* (1998)
Humicola	Chen *et al.* (1998)
T. lanuginosus	Chen *et al.* (1998)
A. oryzae	Chen *et al.* (1998)
Pseudomonas mendocina	Chen *et al.* (1998)
Pseudomonas alcaligenes	Chen *et al.* (1998)
Candida rugosa	Jaeger and Reetz (1998)
C. Antarctica	Sih and Wu (1989) and Vulfson (1994)
C. antarctica	Sih and Wu (1989) and Vulfson (1994)
Acinetobacter radioresistens	Chen *et al.* (1998)
Candida antarctica	Shimada *et al.* (1999)
Pseudomonas cepacia,	Kaieda *et al.* (2001)
Rhizomucor miehei	Robles-Medina *et al.* (2009)
Thermomyces lanuginosus	Robles-Medina *et al.* (2009)

and the physical properties of a triglyceride are greatly influenced by factors like position of the fatty acid in the glycerol backbone, the chain length of the fatty acid and its degree of unsaturation. Lipases allow us to modify the properties of lipids by altering the location of fatty acid chains in the glycerol and replacing one or more of the fatty acids with new ones (Pabai *et al.*, 1995; Undurraga *et al.*, 2001).

Microbial lipases which are regiospecific and fatty acid specific are of enormous importance and could be exploited for industrial vegetable oils. Cheap oils could also be upgraded to synthesize nutritionally important structured triacylglycerols, low calories triacylglycerols and oleic acid enriched oils. Lipase mediated modifications are highly significant in oil industry for the production of structured lipids since enzymatic modifications are specific and can be carried out at moderate reaction conditions (Gupta *et al.*, 2003).

Lipases have also been widely used in food industry to modify flavour by synthesis of esters of short chain fatty acids and alcohols which are known flavour and fragrance compounds (Macedo *et al.*, 2003). Generally, microbial lipases are widely applied in different fields including flavour development for dairy products (cheese, butter, margarine, alcoholic beverages, milk chocolate and sweets), achieved by selective hydrolysis of fat triglycerides to release free fatty acids. These fatty acids can serve as either flavours or flavour precursors.

Lipases are also used to remove fat from meat and fish products (Kazlauskas and Bornscheuer, 1998) to produce lean meat. The fat is removed during the processing of the fish meat by adding lipases and this procedure is called bio-lipolysis. The lipases also play a significant role in the fermentative process of sausage manufacture and to determine changes in long-chain fatty acid liberated during ripening. Earlier, lipases of different microbial origin have been used for refining rice flavour, modifying soybean milk and for improving the aroma and accelerating the fermentation of apple wine (Seitz, 1974).

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* AY30, *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).

Lipases in oil and fat industry: The use of enzymes in the oils and fats industry is new, providing several solutions to both the industry problems and the key to produce novel oils and fats. Lipases can catalyze reactions under mild conditions (i.e., the industrial hydrolysis of fats and oils or the manufacture of fatty acid amides), permitting high specificity; they can therefore be used to obtain high-value chemicals for food and industrial uses at competitive production costs. For example, cocoa butter fat required for chocolate production is often in short supply and the price can fluctuate widely. However, lipase catalyzed transesterification of cheaper oils can be used, for example to produce cocoa butter from palm mid-fraction. The lipase catalyzed transesterification in organic solvents is an emerging industrial application such as production of cocoa butter equivalent, human milk fat substitute, pharmaceutically important Polyunsaturated Fatty Acids (PUFA) and production of biodiesel from vegetable oils (Nakajima *et al.*, 2000). Therefore, lipase-based technology involving mixed hydrolysis and synthesis reactions which are widely used in commercial activity to upgrade some of the less desirable fats to cocoa butter substitutes (Undurraga *et al.*, 2001). One of the application of lipase-based technology is used the immobilized *Rhizomucor miehei* lipase for the transesterification reaction that replaces the palmitic acid in palm oil with stearic acid. Similarly, a lipase-catalyzed interesterification of butter fat was used to decrease the long-chain saturated fatty acids and a corresponding increase in C18:0 and C18:1 acid at position 2 of the selected triacylglycerol (Pabai *et al.*, 1995).

Another example is the use of lipases to enrich polyunsatured fatty acids (PUFAs) from animal and plant lipids. Free PUFAs and their mono-and diglycerides are subsequently used to produce a variety of

pharmaceuticals (anti-inflammatories, thrombolytics, etc.) (Jaeger and Reetz, 1998; Belarbi *et al*., 2000). Because of their metabolic effects, PUFAs are increasingly used as pharmaceuticals, nutraceuticals and food additives (Belarbi *et al*., 2000). Many of the PUFAs are essential for normal synthesis of lipid membranes and prostaglandins. Microbial lipases are used to obtain PUFAs from animal and plant lipids such as menhaden oil, tuna oil and borage oil. In addition, the flavour development for dairy products (cheese, butter, margarine, bakery products, alcoholic beverages, milk chocolate and sweets) is achieved by selective hydrolysis of fat triglycerides to release free fatty acids which act as flavour precursors (Jaeger and Reetz, 1998).

Immobilized *M. miehei* lipase in organic solvent catalyzed the reactions of enzymatic interesterification for production of vegetable oils such as; corn oil, sunflower oil, peanut oil, olive oil and soybean oil containing omega-3 polyunsaturated fatty acids. Lipases are important to hydrolyze lipids so as to obtain fatty acids and glycerol, both of which have important industrial applications. For instance, fatty acids are used in soap production (Hoq, 1985) and glycerol is widely served as raw material for pharmaceutical industries.

Lipases in the detergent industry: 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. 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). Detergent lipases are especially selected to meet the following requirements: (1) a low substrate specificity, i.e., an ability to hydrolyze fats of various compositions; (2) ability to withstand relatively harsh washing conditions (pH 10-11, 30-60°C); (3) ability to withstand damaging surfactants and enzymes (e.g., linear alkyl benzene sulfonates and proteases) which are important ingredients of many detergent formulations. Moreover, the trend towards lower washing temperatures has made the removal of grease spots a bigger problem, particularly for cotton and polyester clothes. Some specific lipases are capable of removing greasy stains such as lipstick, frying fats, butter, sauces, etc. (Jaeger and Reetz, 1998). Enzymes can also reduce the environmental load of detergent since they are biodegradable, leaving no harmful residues; have no negative impact on sewage treatment processes and do not present a risk to aquatic life.

Particularly, 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 use of cold active lipase as a liquid leather cleaner 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 most important lipase in the market was originally obtained from *Humicola lanuginosa*. It is produced in large scale by *Aspergillus oryzae* host after cloning the *Humicola* gene into this organism. Lipolase which originated from the fungus *T. lanuginosus* was also expressed in *A. oryzae*. Lipases isolated from *Pseudomonas mendocina, Pseudomonas alcaligenes* were also used in detergent industry. Alkaline lipase produced by *Acinetobacter radioresistens* had an optimum pH of 10 and was stable over a pH range of 6-10; therefore have great potential for application in the detergent industry (Chen *et al*., 1998). Currently, lipases with the desired properties are obtained through a combination of continuous screening (Yeoh *et al*., 1986; Wang *et al*., 1995; Cardenas *et al*., 2001) and protein engineering (Kazlauskas and Bornscheuer, 1998).

Lipases in pulp and paper industry: 'Pitch' is a term used to collectively describe the hydrophobic components of wood (triglycerides and waxes). Pitch and related substances which are usually creating problems are common in paper mills ((Jaeger and Reetz, 1998). These problems appear as sticky deposits in the paper machines and can cause holes and spots in the final paper. However, lipases are used to remove the pitch from the pulp produced during paper making processes (Jaeger and Reetz, 1998). Lipases hydrolyze up to 90% of triglycerides in the pitch into glycerol/ monoglycerides and fatty acids which are far less sticky and more hydrophilic (easy to wash) (Jaeger and Reetz, 1998). Nippon Paper Industries, in Japan, have developed a pitch control method that uses the *Candida rugosa* fungal lipase to hydrolyze up to 90% of the wood triglycerides (Jaeger and Reetz, 1998).

Generally, the enzymatic pitch control method using lipases have been in use in a large-scale paper-making process as a routine operation since early 1990s

(Bajpai, 1999). Lipases in paper industry can generally increase the pulping rate of pulp, increase whiteness and intensity, decrease chemical usage, prolong equipment life, reduce pollution level of waste water, save energy and time and reduce composite cost. The addition of lipase from *Pseudomonas* species (KWI-56) to a deinking composition for ethylene oxide-propylene oxide adduct stearate improved whiteness of paper and reduced residual ink spots (Fukuda *et al*., 1990).

Lipases in leather industry: Hides and skins contain proteins and fat in the collagen fibres. Before the hides and skins are going tanned, these substances must be partially or totally removed. The first treatment is soaking. This step serves to remove the common salt and free the hide from blood and dirt. At the same time, non-fibril proteins which hold the fibres together have to be eliminated by the action of proteases. Proteolytic enzymes facilitate both the emulsification of natural fat by hydrolyzing the wall of the fat cells and the soaking operation. Lipases specifically degrade fat and do not damage the leather itself. Lipases represent the method of removing fat in the degreasing process with the lowest environmental impact. For bovine hides, lipases allow tensile to be completely replaced. For sheepskins, the use of solvents is very common, but it can also be replaced by lipases and surfactants.

Lipases in organic synthesis: Now days, of those industrially important enzymes, lipases are widely used for organic reactions. They are used to catalyze a wide variety of regioselective and stereoselective transformations (Kazlauskas, 1994; Berglund and Hutt, 2000). Currently, most of lipases used as catalysts in organic chemistry are of microbial origin. These enzymes work at hydrophilic-lipophilic interface and tolerate organic solvents in the reaction mixtures. Use of lipases in the synthesis of enantiopure compounds has been reported by Berglund and Hutt (2000). For instance, *Pseudomonas* lipases are widely used in industry, especially for the production of chiral chemicals which serve as basic building blocks in the synthesis of pharmaceuticals, pesticides and insecticides. These enzymes show distinct differences in regioselectivity and enantioselectivity, despite a high amino acid sequence homology.

Generally, lipases have become one of the most important groups of enzymes for its applications in organic syntheses. Lipases are used as biocatalyst in the production of significant biodegradable compounds. Trimethylolpropane esters were synthesized as lubricants. Lipases can catalyze ester syntheses and transesterification reactions in organic solvent systems has opened up the possibility of enzyme catalyzed production of biodegradable polyesters. Aromatic polyesters can also be synthesized by lipase biocatalysis (Bailey and Ollis, 1986).

Lipases in textile industry: Lipases are widely used in the textile industry to remove size lubricants and thereby to provide a fabric with greater absorbency for improved levelness in dyeing. It is also used to reduce the frequency of streaks and cracks in the denim abrasion systems. Lipases together with alpha amylase are used for the desizing of denim and other cotton fabrics at commercial scale (Rowe, 2001).

In the textile industry, polyester has certain key advantages such as it increases strength, soft hand, stretch resistance, stain resistance, machine wash ability, wrinkle resistance and abrasion resistance. Synthetic fibers have been processed and modified by the action of enzymes for the use in the production of yarns, fabrics, textiles, rugs and other consumer items. It relates to modification of the characteristics of a polyester fiber as the result that such polyesters are more susceptible to post-modification treatments. The use of poly-esterase (closely related to lipase) can improve the ability of a polyester fabric to uptake chemical compounds, such as cationic compounds, fabric finishing compositions, dyes, anti-static compounds, anti-staining compounds, antimicrobial compounds, antiperspirant compounds and/or deodorant compounds (Rowe, 2001).

Lipases in the production of cosmetics: Some cosmetic industries are currently produced isopropyl myristate, isopropyl palmitate and 2-ethylhexyl palmitate for use as an emollient in personal care products such as skin and sun-tan creams, bath oils etc. In this case, immobilized *Rhizomucor miehei* lipase was widely used as a biocatalyst. The use of the enzyme instead of the commonly used acid catalyst gives products of much higher quality with minimum downstream refining process.

Wax esters (esters of fatty acids and fatty alcohols) have similar applications in personal care products and are also being manufactured by the action of lipase produced from *C. cylindracea* using batch bioreactor. The overall production cost in this way is slightly higher than that of the conventional method used, however the cost is compensated by the improved quality of the final product. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase (Maugard *et al*., 2002). Lipases have been used in hair waving preparation

(Saphir, 1967). Lipases have also been used as a component of topical anti-obese creams (August, 1972) or as oral administration (Smythe, 1951).

The role of lipases in medical and pharmaceutical application: Currently, lipases are widely used in medical and pharmaceutical industry. For instance, enantioselective interesterification and transesterification reaction by the help of lipases have great significance in pharmaceutical industry for selective acylation and deacylation reaction (Stinson, 1995). Lipases 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 stereo-preference. Lipases play an important role in modification of monoglycerides for use as emulsifiers in pharmaceutical applications (Sharma *et al*., 2001a).

Lipase from *Candida rugosa* has been used to synthesize lovastatin, a drug that lower serum cholesterol level. *S. marcescens* lipase was widely used for the asymmetric hydrolysis of 3-phenylglycidic acid ester which is a key intermediate in the synthesis of diltiazem hydrochloride (Matsumae *et al*., 1993).

The application of lipases in 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 (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 the technological processes using cold active lipase from *C. antarctica* have been patented by chemical, food industries and pharmaceutical industries.

Lipases in biodiesel production: Biodiesel is a liquid biofuel which are esters of long chain fatty acids and short chain alcohols. Biodiesel molecules are synthesized through direct transesterification of vegetable oils and fats with short chain alcohols (such as methanol and ethanol) in the presence of suitable catalysts (Fig. 2) (Vicente *et al*., 2004).

Transesterification is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is employed instead of water (Srivastava and Prasad, 2000). Among short chain alcohols, methanol and ethanol are usually used, especially, because of its low cost and physicochemical advantages, methanol is used frequently. This process has been widely used to reduce the viscosity of triglycerides, thereby enhancing the physical properties of renewable fuels to improve engine performance (Clark *et al*., 1984).

The main factors affecting transesterification are molar ratio of triglycerides to alcohol, catalysts, reaction temperature and time, the contents of free fatty acids and water in oils and fats (Freedman *et al*., 1986). Biodiesel catalysts are currently, classified as alkali, acid, or enzyme. This review is only focused on the production of biodiesel using lipase as catalyst.

For the production of biodiesel, an alkali-catalysis process has been established that gives high conversion levels of oils to methyl esters. However, it has several drawbacks, including the difficulty of purifying glycerol and the need for either removal of the catalyst or wastewater treatment. In particular, several steps such as the evaporation of methanol, removal of saponified products, neutralization and concentration, are needed to recover glycerol as an added value product.

To overcome these drawbacks which may limit the availability of biodiesel fuel; enzymatic processes using lipase have recently been developed. Since the cost of lipase production is the main hurdle to the commercialization of the lipase-catalyzed process, the use of intracellular lipase or cell-surface-displayed lipase as a whole-cell biocatalyst through the application of immobilization techniques (Ban *et al*., 2002) has been considered as an effective way to lower the lipase production cost. Unlike in the case of extracellular lipase, these whole-cell biocatalysts can be prepared by simple cultivation and recovered easily.

However, to utilize these whole-cell biocatalysts for industrial application, a repeated methanolysis reaction cycle is required in order to produce high methyl ester content of 90-95%. One potential solution is the use of a whole-cell biocatalyst possessing a non-specific lipase from a source such as *Candida antarctica* (Shimada *et al*., 1999) or *Pseudomonas cepacia* (Kaieda *et al*., 2001) within the cell or on the cell-surface, since these lipases realize methyl ester content of more than 95%. Such a system could offer a promising prospect of realizing industrial biodiesel fuel production. However, bodiesel production by lipase is not yet commercialized.

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