Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (1): 1-24, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.1.24

Bacterial Lipases as Potential Industrial Biocatalysts: An Overview

[1]R. Sangeetha, [2]I. Arulpandi and [3]A. Geetha
[1]Department of Biochemistry, School of Life Sciences, Vels University, Chennai-600 117, India
[2]Department of Microbiology, Asan Memorial College, Chennai-600 100, India
[3]Department of Biochemistry, Bharathi Women's College, Chennai-600 108, India

Corresponding Author: R. Sangeetha, Department of Biochemistry, School of Life Sciences, Vels University, Pallavaram, Chennai-600 117, India Tel: +919884204394

ABSTRACT

Lipases are hydrolytic enzymes which hydrolyse triglycerides to free fatty acids and glycerol. These enzymes also catalyse transesterification, aminolysis and acidolysis reactions. Their potential to function in non-aqueous and micro-aqueous environments makes them a versatile biotechnological tool. Lipases are ubiquitous in nature and those from microbes have occupied a prominent position as industrial biocatalysts. Both fungal and bacterial lipases have been exploited by various industries. The chemo, regio and enantio-selectivity of lipases are properties which widen their range of industrial applications. This review was aimed at providing a collective knowledge about bacterial lipases and this article discusses the fundamental details about the sources and cellular location of lipase and the production and purification of the enzyme. Immobilisation and bioimprinting techniques which improve the catalytic efficacy of lipase have also been discussed. This overview also enumerates and describes briefly the extensive applications of bacterial lipases.

Key words: Bacterial lipases, industrial applications, production, purification, characterisation

INTRODUCTION

Man has been using enzymes for ages, in different forms, as extracts obtained from vegetables or animal organs or as microbes. The history of modern enzyme technology can be dated from the late 19th century when the Danish chemist Christian Hansen produced rennet by extracting dried calves' stomachs with saline solution. This was followed by extensive research on the resources and applications of enzymes. The industrial biotechnology sector experienced a major breakthrough when it was understood that enzymes could be exploited commercially. A large number of enzymes are being produced and sold for various purposes and the blooming industrial enzyme market is one of the major revenue generators in the life sciences-industry sector. Global Industry Analysts Inc., have reported a comprehensive analysis on worldwide market for industrial enzymes. According to this global strategic business report, the world market for enzymes would exceed $2.9 billion by 2012 and the major product segments include carbohydrases, proteases and lipases (www.reportlinker.com/World-Industrial-Enzymes-Market).

Lipase (triacylglycerol acylhydrolase, EC. 3.1.1.3) is an important hydrolytic enzyme with innumerable applications and industrial potential. Lipids are major macromolecules which play significant physiological roles and lipolytic enzymes are required for their turnover. Lipolytic enzymes include esterases commonly called carboxyesterases (EC. 3.1.1.1) and lipases originally

called true lipases. The fundamental striking difference between esterases and lipases is that the former acts on short chain triglycerides which are soluble in water. Lipases, however, act on lipids which form aggregates in water and require a water-lipid interface for its catalysis. Lipases can hydrolyze long chain water-insoluble triglycerides into diglycerides, monoglycerides, glycerol and fatty acids (Gilham and Lehner, 2005; Angkawidjaja and Kanaya, 2006). Bacterial lipases cannot be well distinguished as lipolytic and esterolytic enzymes despite many attempts by researchers to classify them (Rosenstein and Gotz, 2000).

Lipases are ubiquitous enzymes which are widely distributed in plants, animals and microbes (Dutta and Ray, 2009). Lipases have gained special attention over few decades owing to their ability to act in micro-aqueous environment and catalyse esterification, trans-esterification, aminolysis, acidolysis reactions (Joseph *et al*., 2008). Many industrial applications of lipase focus on its regio-and enantio-selectivity properties (He *et al*., 2010). Also, lipases do not require cofactors to catalyse hydrolytic reactions and remain active in the presence of organic solvents. Lipases demand enormous attention these days because of these above said properties. Therefore, lipase has become a prime focus of enzymologists and few extensive interesting reviews are available on its classification (Arpigny and Jaeger, 1999), assay and detection methods (Gilham and Lehner, 2005; Hasan *et al*., 2009), purification strategies (Saxena *et al*., 2003) and industrial applications (Hasan *et al*., 2006).

Lipases have been isolated from a large number of plant, animal and microbial sources. The ease, with which enzymes could be isolated from microbes, has made both bacteria and fungi as predominant sources of lipase. This review reports on the production, purification, characterization and applications of bacterial lipases.

HISTORY OF LIPASES

Lipase was first discovered in pancreatic juice in the year 1856 by Claude Bernard. Animal pancreatic extracts were traditionally used as the source of lipase for commercial applications. However, microbial sources of lipase were explored when the industrial potential of lipases enhanced and when the demand for lipases could not be met by the supply from animal sources.

Bacterial sources: Microbial sources are superior to plants and animals for enzyme production and this can be attributed to the ease with which they can be mass cultured and genetically manipulated (Hasan *et al*., 2006). Commercial microbial lipases are produced from bacteria, fungi and actinomycetes (Babu and Rao, 2007). Lipases isolated from fungi are best studied among all microbial lipases. Nevertheless, bacterial strains are being constantly screened and improved for lipase production. Bacterial lipases were first observed in the year 1901 in the strains *Serratia marescens* and *Pseudomonas aeruginosa* (Hasan *et al*., 2006). Ever since that lipase production by many different bacterial species has been extensively studied and reported. There are various documents available on the production of bacterial lipases particularly from *Pseudomonas* and *Bacillus* sp., *P. aeruginosa* (Madan and Mishra, 2010). *Pseudomonas fluorescens* (Yang *et al*., 2009), *Bacillus pumilus* (Sangeetha *et al*., 2010a), *B. thermocatenulatus* (Quyen *et al*., 2003), *B. subtilis* (Ahmed *et al*., 2010), *B. licheniformis* (Sangeetha *et al*., 2010b), *B. coagulans* (Mnisi *et al*., 2005), *B. cereus* (Dutta and Ray, 2009) and *B. halodurans* (Ramchuran *et al*., 2006). Other genera like *Acinetobacter* (Li *et al*., 2004), *Staphylococcus* (Talon *et al*.,1996), *Streptococcus* (Tripathi *et al*., 2004), *Burkholderia* (Wang *et al*., 2009), *S. marescens* (Long *et al*., 2007), *Achromobacter*, *Arthrobacter*, *Alcaligenes* and *Chromobacterium* (Riaz *et al*., 2010) too have been studied.

Cellular location of lipases: Bacterial lipases may be intracellular, membrane-bound or extracellular. A *B. clausii* strain which produces only intracellular lipase has been reported (Lee and Park, 2008). Strains which produce only intracellular lipase can grow only on glycerol and simple lipids but not on long chain triglycerides. Ertugrul *et al.* (2007) have observed the production of both intracellular and extracellular lipase in *Bacillus* sp. Boekema *et al.* (2007) have reported the production of extracellular lipase as consequence of secretion of accumulated intracellular lipase by membrane-bound chaperones.

Bacteria secrete lipase to the external medium through different types of secretory systems. The type I Secretory System (T1SS) comprises of an energy driven exporter complex made up of three protein subunits. The type II Secretory System (T2SS) has two components-the (general protein secretion) Sec-dependent pathway and the (twin-arginine translocation) Tat-dependent pathway. Bacterial lipases are secreted in the unfolded state via the Sec-dependent pathway into the periplasmic space where folding takes place with the assistance of chaperone called lipase-specific foldase (Lif). The folded lipases are then transported out into the external medium by a transporter complex (Angkawidjaja and Kanaya, 2006; Buist *et al.*, 2006).

Classification of bacterial lipases: Lipases belong to the family of serine hydrolases and their activity relies on a catalytic triad comprising of serine, histidine and aspartate and an α/β hydrolase fold (De Pascale *et al.*, 2008). Bacterial lipolytic enzymes were classified into 8 families and the largest family was subdivided into 6 sub-families by Arpigny and Jaeger (1999). This classification was based on the conserved sequence motifs and biological properties of the enzymes. True lipases belong to family I which comprises most of the *Pseudomonas*, *Bacillus* and *Staphylococcus* lipases. These lipases possess the conventional catalytic pentapeptide Gly-Xaa-Ser-Xaa-Gly. Family II lipases exhibit Gly-Asp-Ser-Leu motif at the active site and esterases of *Streptomyces*, *Aeromonas* and *Salmonella* belong to this family. Family III comprises of lipases of *Streptomyces* sp but unlike family II esterases these are extracellular lipases. Lipases which display similarity with mammalian hormone sensitive lipases are grouped under family IV while lipases of mesophilic bacteria like *P. oleovorans* and *Haemophilus influenza* belong to family V. Family VI lipases are the smallest esterases and the active enzymes are dimeric. Family VII lipases are large esterases and their amino acid sequence is homologous to that of eukaryotic acetyl choline esterases. Family VIII lipases are similar to β-lactamases. The sequences of few other enzymes could not be grouped into any of the eight super families described by Arpigny and Jaeger and have been arbitrarily classified as new family 9 and 10. A cold active lipase reported by De Pascale *et al.* (2008) could not fit into the traditional classification and hence reported as a lipase belonging to a novel lipolytic family.

MELDB is another comprehensive database of microbial lipases and esterases (Kang *et al.*, 2009). The orphaned lipases which do not belong to any of the eight superfamilies but arbitrarily grouped with them in the traditional classification have been out-grouped in the MELDB database. This classification was done with conserved sequences of enzymes based on a local sequence alignment and a graph clustering algorithm (Tribe MCL).

PRODUCTION OF LIPASES

Screening of lipase production: Several methods have been proposed for screening of lipase production. These methods either directly use the microorganism under study (Nair and Kumar, 2007) or measure lipolytic activity in the crude or purified culture preparations (Singh *et al.*, 2010).

The plate detection methods use agar plates containing lipid substrate and lipolysis was observed as clear halos or opaque zones around the well containing culture or enzyme preparation (Hun *et al*., 2003; Nair and Kumar, 2007). Plates containing chromogenic substrates with pH indicators like phenol red, Victoria blue etc are also used (Singh *et al*., 2006; Rahman *et al*., 2005). A decrease in pH due to release of fatty acids due to lipolysis causes change in the colour of the indicators is measured. Rhodamine B plates which indicate lipolysis by the formation of fluorescent orange halos are widely used (Kim *et al*., 2001). The colorimetric methods are based on measuring the complexes formed between the released free fatty acids and a divalent metal ion, usually copper (Rahman *et al*., 2005). The widely adopted spectrophotometric methods use designed substrates usually p-nitrophenyl esters of fatty acids and the lipolytic activity is assayed by measuring the amount of p-nitrophenol released after to the process (Wang *et al*., 2009). The titrimetric method employs the neutralisation of the free fatty acids that are released after lipolysis and the volume of the base consumed indicates the extent of lipolysis (Shukla *et al*., 2007). The other least commonly used methods like chromatographic, turbidimetric, fluorimetric, immunological and radioactive assays are best elaborated in a review on detection methods by Hasan *et al*. (2009).

Production media: Bacterial lipases are produced by both submerged (Chakraborty and Paulraj, 2008) and solid-state fermentation (Alkan *et al*., 2007). Lipase production requires carbon and nitrogen sources as required by any fermentation process. Most of the lipase production studies do not use simple sugars as carbon sources rather use lipid substrates as sole carbon sources (Zhang *et al*., 2009a, b). However, few studies utilise sugars like glucose as carbon sources while lipid substrates are considered as inducers for lipase production (Hun *et al*., 2003). Lipase production is rarely constitutive and the quantity of the extracellular lipase produced is meagre (Lee *et al*., 2001). Hence inducers like vegetable oils (Kumar *et al*., 2005), Tween 20/80 (Li *et al*., 2004), hexadecane (Boekema *et al*., 2007) and synthetic triglycerides like tributyrin and tripalmitin (Rahman *et al*., 2006) are used. High concentrations of free fatty acid or vegetable oil repress lipase synthesis. Hence, many lipase production studies use Tween 80 as a sole carbon source. Moderate and sustained release of oleic acid from Tween 80 does not cause repression (Li *et al*., 2004). Nitrogen sources have varied effect on lipase production and sources like peptone have been reported to augment lipase production (Gunasekaran *et al*., 2006). Unlike carbon sources, nitrogen sources have not been reported to repress lipase production.

The presence of surfactant is an important pre-requisite for maximum lipase production. Catalytic activity of lipases is governed by interfacial activation, a property observed when the lipid substrate starts to form an emulsion thereby presenting an interface for the enzyme to act. Addition of a surfactant decreases the surface tension between the organic and aqueous phase present in the reaction mixture and enhances the rate of emulsification (Wu and Tsai, 2004). The commonly used surfactants are Tween, Triton-X 100 (Pogaku *et al*., 2010).

PURIFICATION

Downstream processing is fundamental for any fermentation process and involves isolation and purification sequences to obtain a pure and homogenous product (Nandini and Rastogi, 2009). Since commercial lipases are generally extracellular, the purification processes are easy though extensive. The cell-free supernatant which is considered as the crude enzyme source is subjected to preliminary purification steps like ultrafiltration (Quyen *et al*., 2003), spray-drying using milk powder or gum arabic (Fickers *et al*., 2006), ammonium sulphate fractionation (Kim *et al*., 2002)

and/or precipitation using ice-cold organic solvents like ethanol, ether and acetone (Chakraborty and Paulraj, 2008). This initial preparative purification is followed by purification using a combination of chromatographic techniques. A single chromatographic purification step will usually not be sufficient to get an enzyme of high purity (Saxena *et al*., 2003). The commonly reported chromatographic methods to purify lipase are Ion Exchange (IEC), Gel Filtration (GFC) and Hydrophobic Interaction Chromatography (HIC). The IEC is the most commonly employed chromatographic method and the frequently used ion exchangers are Q-Sepharose (Nawani and Kaur, 2000), DEAE-Sepharose (Kumar *et al*., 2005) and CM-cellulose (Vujaklija *et al*., 2003). GFC, the next best adopted method uses matrices like Sephacryl (Sharma *et al*., 2002), Sephadex (Lee *et al*., 2001). HIC using octyl or phenyl adsorbents is adopted for lipase purification (Saxena *et al*., 2003). Nawani and Kaur (2007) have employed IEC, GEC and HIC to purify two lipase isoenzymes from a *Bacillus* sp. Affinity chromatography using Ni^{2+}-chelated nitriloacetic acid (Ni-NTA) agarose column is also used by many researchers (Zhang *et al*., 2009a, b; Akbari *et al*., 2010).

Pauwels and Gelder (2008) have devised a new strategy to purify lipase and this method exploits the principle behind lipase secretion mechanism. Bacterial lipases, particularly those belonging to the family I are secreted in an unfolded state in to the periplasm where they are folded and conferred the native conformation by an inner membrane bound protein called lipase-specific foldase (Lif). Lif has high affinity for lipase and exhibits high specificity (Rosenau *et al*., 2004; Pauwels and Gelder, 2008). Purification of lipase was performed using immobilised His-tagged Lif. The purified lipase-Lif complex was homogenous and was devoid of the lipopolysaccharides which are usually found as a contaminant in lipase preparations obtained by conventional purification procedures. da Silva (Padilha *et al*., 2009) have purified an alkaline lipase from *P.cepacia* using Expanded Bed Absorption (EBA) on Amberlite ion-exchange resin. EBA is a novel technique which can purify crude enzyme preparations without subjecting them to initial preliminary purifications processes. Aqueous Two-Phase System (ATPS) extraction is another principal downstream processing method which promises lipase purification in less time than other conventional methods. ATPS employs two phases which are immiscible beyond a particular concentration and may be polymer/polymer or polymer/salt or organic solvent/salt systems (Ooi *et al*., 2009).

IMMOBILIZED AND NANO LIPASES

Use of enzymes as industrial catalysts serves to be beneficial if the whole process is economical and the cost of any process involves the production of the biocatalyst also. Hence recovery of the catalysts for repeated use becomes necessary. Free enzymes are labile and vulnerable to degradation during the process of recovery of the used enzyme. Also, most lipases exhibit low stability and activity in organic media (Lee *et al*., 2010). These disadvantages could be overcome by the use of immobilized enzymes. Immobilization improves the stability of enzyme under the reaction conditions, enhances enzyme activity thus, makes the repeated use of the enzyme feasible, permits the use of enzyme for diverse applications and thus lowers production costs (Guncheva *et al*., 2009; Liu *et al*., 2009). Immobilisation provides a better environment for the enzyme to act and also offers better product recovery (Lee *et al*., 2009).

Lipase may be immobilized on different kinds of hydrophobic or hydrophilic supports (Minovska *et al*., 2005). Various support materials like ion-exchange resins (Amberlite IRC-50), inorganic materials (sand, silica), biopolymers namely sodium alginate (Cheirsilp *et al*., 2009), synthetic polymers like polypropylene, polyethylene, polymethacrylate (Salis *et al*., 2009), celite

(Liu *et al*., 2009), ceramic (Al-Zuhair *et al*., 2009), complexes like polypropyleneimine-agarose were used to immobilize lipase. Hydrophobic silicates derived from a mixture of TMOS and alkyltrimethoxysilanes such as propyltrimethoxysilane (PTMS) and *n*-butyltrimethoxysilane (BTMS) were identified as best supports for lipase (Furukawa *et al*., 2002). Mesoporous silica materials with high surface area and ordered pore structures have been used to immobilize lipase and the immobilized enzyme was found to be more efficient and stable than the free enzyme (Kato and Seelan, 2010).

Mesoporous materials meet all the criteria for a perfect immobilization support-water insolubility, high surface area, mechanical strength, chemical stability, thermo tolerance and non-toxic (Hartmann, 2005; Jaladi *et al*., 2009). Ramani *et al*. (2010) have reported the use of mesoporous activated carbon derived from rice husk as carrier for immobilizing lipase. Protein-Coated Micro Crystals (PCMC) have been extensively for enzyme immobilization in the recent years. PCMC are produced by mixing the enzyme with an excipient and the mixture when added to a water-immiscible solvent precipitates and microcrystals of the excipient forms on whose surface the enzyme gets immobilized. Ruchi *et al*. (2008a) have prepared PCMC of lipase from *P. aeruginosa* and found to be stable and efficient. Devi *et al*. (2009) have immobilized lipases as cross-linked enzyme aggregates using precipitants and cross-linkers. These enzyme aggregates do not require pre-existing carriers and can function as effective catalysts under both aqueous and non-aqueous conditions.

Magnetic nano particles possess important advantages when compared to porous supports; nano size of the support particles provide high surface area and thus maximum enzyme loading, low diffusion of the enzyme in to the support and the immobilized enzyme could be controlled and recycled by applying magnetic field (Hu *et al*., 2009). Lee *et al*. (2009) have suggested the use of nano-sized magnetite particles for immobilizing lipase. Low molecular weight ligands were used to adsorb the lipase on to the surface of the nano particles. Carbon nano tubes have found extensive applications as biosensors and nano-biocatalysts owing to their application as immobilizing support for enzymes and have been demonstrated as perfect supporters for lipase immobilization (Lee *et al*., 2010). Electrospun nanofibres have proved to be excellent immobilization supports and lipase has been immobilized by physical adsorption on to electrospun nanofibres (Sakai *et al*., 2010).

BIOIMPRINTING

Bioimprinting is a convenient method to improve the catalytic performance of lipases. This method was developed to enhance lipase activity and stability in non-aqueous mediums, particularly in organic solvent and solvent-free systems. The technique of bioimprinting is based on induction of reversible changes in the three dimensional structure of lipase by noncovalent interactions with a ligand using lyophilization and an anhydrous solvent. Lipase exists in two conformational states: a closed, inactive state and an open, active state. The catalytic site is closed by a helical lid in the former while the active site is exposed in the latter by the displacement of the lid in response to inducing agents or substrates (Mingarro *et al*., 1995). The lid movement also shapes the catalytic machinery to form an oxyanion hole (Yilmaz, 2003). The induced conformation change of lipase thus presumably involves opening of its lid structure and shaping of the catalytic machinery. In bioimprinting, the conformational change is induced by imprint molecules which are amphiphiles, substrates or substrate analogues. While, rapid freeze-drying helps in trapping lipase in its open and active conformation, washing with anhydrous solvents removes the imprint molecules. The commonly used imprint molecules are Tween 20, olive oil (Yilmaz, 2002), fatty acids

(Yan *et al*., 2010) and Orlistat, a reversible lipase inhibitor (Yilmaz, 2003). Sol-gel encapsulation has been reported as a simple process which utilises imprint molecules and silane precursor for bioimprinting (Cao *et al*., 2009).

BIOCHEMICAL CHARACTERISTICS OF BACTERIAL LIPASES

The biochemical characterisation of any enzyme becomes indispensable to understand its requirements to exhibit its maximal catalytic performance, which in turn is necessary for the best industrial exploitation of that enzyme. The characteristics that are usually studied are the optimal pH and temperature of enzymes, influence of the presence of cofactors, inhibitors and enhancers on catalytic activity, tolerance of the enzyme to organic solvents and proteases.

Acidic and alkaline lipases: Bacterial lipases are mostly alkaline in nature and alkaline lipases are the promising catalysts for many industrial processes (Nawani and Kaur, 2007; Ahmed *et al*., 2009). Despite many reports on lipases and lipase producing microbes, the documents on acidic lipases are very few. Those few, however report on acidic lipases from fungi, especially *Aspergillus niger*, except for a study by Ramani *et al*. (2010) who investigated the production of acidic lipase by *P. gessardii*.

Thermophilic and cold adapted lipases: Thermal stability is a desirable feature of an industrial enzyme which is employed in processes that require temperatures greater than equal to 60°C. Lipases are most preferred if they are thermostable. This is because of the high temperatures employed in lipolytic reactions, mainly because of the high melting point of the lipidic substrates participating in the process. Thermostable lipases from many *Pseudomonas* and *Bacillus* sp. have been isolated and studied (Nawani and Kaur, 2000; Kumar *et al*., 2005; Ahmed *et al*., 2009; Dutta and Ray, 2009). The spectrum of the industrial potential of cold adapted lipases encompasses a wide range of biotechnological applications. These enzymes exhibit high catalytic activity at temperatures between 0 and 30°C (Cai *et al*., 2009) and are generally produced by psychrophilic microorganisms which survive at temperatures around 5°C (Joseph *et al*., 2008). Cai *et al*. (2009) have reported cold-adapted lipases from a mesophilic *Geotrichum* sp.

Effect of detergents/surfactants: Surfactants increase the lipid-water interface and thus enhance the rate of lipolysis. However, this does not hold true for all surfactants. Moreover, the effect of surfactants is concentration dependent. For instance, high concentrations of Tween-80 (1%) inhibited lipase production by *B. pumilus* while at 0.5% concentration Tween-80 assisted maximum lipase production (Zhang *et al*., 2009a, b). SDS was found to exhibit inhibitory effect on lipases while Triton X-100 and Tween enhanced reaction rates (Quyen *et al*., 2003; Lianghua and Liming, 2005). The reversal of this is also plausible; (Dutta and Ray, 2009) have observed that SDS exhibited stimulatory effect while Triton and Tween inhibited lipase activity.

Dependence on metal ions: Metal ions enhance the catalytic activity of enzymes and also confer thermostability to them (Chakraborty and Paulraj, 2008). Many enzymes require the presence of metal ions for the maintenance of their active structures (Sharma *et al*., 2002). Different lipases show different response to these metal ions and those ions which function as activators for certain lipases inhibit the activity of few others. The commonly studied metal ions are Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Hg^{2+}, Cu^{2+}, Fe^{2+} etc. Many metal-dependent/metallo lipases have been reported and Ca^{2+}

has been found to be exhibit a stimulatory effect in all those enzymes (Chakraborty and Paulraj, 2008; Ahmed *et al*., 2009; Zhang *et al*., 2009a, b). This effect could be attributed to the structural alterations imposed by the binding of Ca^{2+} to the enzyme. The folded enzyme harbours a region of negatively charged amino acid residues which try to move apart to reduce the electrostatic repulsions and this proves to be detrimental to the stability of the enzyme. The metal ion, however, binds to the enzyme and forms a bridge that cross-links the polypeptide chain (similar to a disulphide bond) and the enzyme-metal ion complex becomes stable and rigid. On the contrary, lipases which exhibit Ca^{2+} independent thermostability and activity also have been reported (Kim *et al*., 2002). Calcium-independent lipases can function effectively in the presence of chelating agents like EDTA usually found in the laundry detergents.

Tolerance to organic solvents: Organic solvents are advantageous than their inorganic counterparts or water when used in reaction systems involving biocatalysts. They help in increasing the solubility of substrates, easy recovery of products and assist in shifting the equilibrium in the forward direction in synthetic reactions (Zhang *et al*., 2009a, b). Organic solvent tolerant-lipases act as effective catalysts in the synthesis of biopolymers, transesterification reactions and production of biodiesel (Dizge *et al*., 2009; Singh *et al*., 2010). The stability and activity of lipase is usually tested in the presence of organic solvents like isopropanol, methanol, ethanol, acetone, glycerol, n-hexane, n-heptane, n-octane, n-decane, benzene, toluene, xylene, styrene, benzene, ethylbenzene, cyclohexane, dimethylsulfoxide, tetrahydrofuran, chloroform and acetic acid (Cadirci and Yasa, 2009; Zhang *et al*., 2009a, b). The sensitivity of lipases to solvents varies depending on their polarity with polar solvents being more destabilizing than non-polar solvents (Ahmed *et al*., 2010). Lipases from different sources exhibit different extents of tolerance to various organic solvents and thus commercial exploitation of lipase demands a thorough analysis of tolerance against these solvents.

Tolerance to proteases: Lipases have a wide range of applications of which few employ lipase in combination with other enzymes like amylase and protease. Proteases are hydrolytic enzymes which are capable of auto-digestion and also digest other enzymes produced simultaneously (Aguilar *et al*., 2002). A thorough study of literature reveals several reports which state that the production of lipase and protease are inter-related (Rajmohan *et al*., 2002). These studies have shown that when the production of protease is affected either due to the influence of production parameters or due to genetic alterations, the production of lipase is enhanced. For instance, (Lopes *et al*., 2008) have proved that the increased air pressure during fermentation decreased protease production and thereby enhanced lipase production. Westers *et al*. (2005) have investigated on the susceptibility of a lipase produced by *B. subtilis* to degradation by extracytoplasmic proteases located in the cell wall or in the growth medium. Nevertheless, there are few documents on proteolysis-resistant lipases. Zhang *et al*. (2008) and Dutta and Ray (2009) have produced a lipase from *Streptomyces fradiae* and *B. cereus* respectively which was resistant to commercial neutral and alkaline proteases. Lipases produced by *P. aeruginosa*, *B. pumilus* and *B. licheniformis* were found to be resistant to co-produced native proteases (Ruchi *et al*., 2008b; Sangeetha *et al*., 2010a, b).

RECOMBINANT BACTERIAL LIPASES

Recombinant DNA technology permits to understand the binding and catalytic site of lipases, overexpress lipases in appropriate hosts to meet commercial demands and to engineer the enzyme.

ipases obtained from culture supernatants suffer various disadvantages like non-reproducibility of results, undesirable side-effects and demand tedious purification processes. Recombinant lipases however, overcome all these constraints and help in large scale production of pure lipases which may or may not be tailor-made (Schmidt-Dannert, 1999). The success of this technology lies in both the choice of the expression system employed for overexpression and the thorough knowledge on the genetic modifications to be made. Cloning and sequencing of lipase genes has been an area of study attracting attention from researchers worldwide. Many bacterial lipases have been cloned, sequenced and expressed in homologous or heterologous hosts. The popular expression host is *Escherichia coli* (An *et al*., 2003; Long *et al*., 2007), while other efficient hosts include *Pichia pastoris* and *Saccharomyces cerevisiae* (Ramchuran *et al*., 2006; Mormeneo *et al*., 2008).

The high-level expression of lipase in *E. coli* often results in formation of insoluble and inactive inclusion bodies (Akbari *et al*., 2010). The overexpressed proteins may not be efficiently processed by the post-translational machinery of the host and this result in protein mis-folding. Dictated by the cell's degradation capacity, the misfolded proteins may be degraded or form insoluble aggregates called inclusion bodies in the cytoplasm or periplasm (Goldberg, 2003). These inclusion bodies have to be solubilized and refolded to recover the active protein. Refolding protocols are being standardized constantly and these protocols use refolding enhancers which are low molecular weight additives. Osmolytes are effective additives and these include polyols, sugars, polysaccharides, amino acids and neutral polymers. Akbari *et al*. (2010) have optimized the refolding conditions of a recombinant lipase from *Pseudomonas* sp. using response surface methodology. Conventional methods use only chemical additives to assist enzyme refolding. The role of lipase-specific foldase (Lif), the chaperones which assist in lipase secretion has been well explored. These proteins can assist in lipase folding both *in vitro* and *in vivo*. The gene encoding Lif was cloned with the lipase coding gene in the same plasmid and evaluated for enhanced expression of lipase in *E. coli*. The yield of lipase was low and this result was attributed to complex gene regulatory and secretion mechanisms. Alternatively, the genes coding lipase and Lif were cloned in separate expression vectors and expressed in different hosts. Lipase expression was found to increase but as inclusion bodies (An *et al*., 2003). In a different attempt, the foldase can be isolated and used along with chemical aids to enhance the protein refolding yield. Akbari *et al*. (2010) adopted this strategy to refold a *Pseudomonas* lipase expressed as inclusion bodies in *E. coli* and observed several fold increase in the refolding yield over the conventional method.

Surprisingly, lipases from *P. fragi* IFO 34584, *P. fluorescens* C9 and *P. fluorescens* JCM 5963 have been expressed in enzymatically active forms in *E. coli* (Zhang *et al*., 2009a, b).

APPLICATIONS OF BACTERIAL LIPASES

Lipases obtained from fungal sources were thought to be the perfect candidates for commercial applications until bacterial lipases were explored. Many microbial lipases have been commercialized by popular enzyme producers in the world like Novozyme (Denmark), Amano Enzyme Inc (Japan), Biocatalysts (UK), Unilever (Netherlands) and Genencor (USA). Bacterial lipases produced form the genera *Burkholderia* and *Pseudomonas* are commercially available. Lipase PS isolated from *Burkholderia cepacia* and Lipase AK isolated *P. fluorescens* are supplied by Amano and Lipase SL and Lipase TL isolated from *B. cepacia* and *P. stutzeri* are supplied by Meito Sangyo (Japan).

Lipases are valuable biocatalysts with diverse applications. Though lipases share only 5% of the industrial enzyme market, they have gained focus as biotechnologically valuable enzymes. They play vital roles in food, detergent and pharmaceutical industries.

Lipases catalyse the hydrolysis of lipids to fatty acids and glycerol. The reversal of this reaction takes place in an environment with low water content or rather in the presence of organic solvents. Such reactions include esterification, transesterification and interesterification. The term interesterification has a broader sense throughout the literature and refers to exchange of acyl radicals between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis) or between two esters (transesterification). All these reactions serve to alter the physical properties of the oil or fat and produce new esters (Ghazali *et al.*, 1995). These reactions provide a platform for lipase to act as a potential industrial enzyme.

Food industry

Hydrolysis of oils: Hydrolysis of oils is usually performed to concentrate the fatty acids present in the oil. The production of a number of high-value products like adhesives, cosmetics and other personal care products, lubricants and coatings requires fatty acids. Thus production of fatty acids by the hydrolysis of oils and fats becomes indispensable for them to be exploited by various industries (Murty *et al.*, 2002). Ramani *et al.* (2010) used an immobilized acidic lipase from *P. gessardii* for olive oil hydrolysis.

The Polyunsaturated Fatty Acids (PUFAs) are simple lipids with two or more double bonds and play diverse physiological roles which contribute to the normal healthy life of human beings. PUFAs belong to two major families namely n-3 (ω3) and n-6 (ω6). The nutritionally important ω3 fatty acids include α-Linolenic Acid (ALA), Eicosatrienoic Acid (ETA), Eicosapentaenoic Acid (EPA) and docosahexaenoic acid (DHA). Linoleic acid and arachidonic acid are the most common ω6 fatty acids. These are currently in high demand as they are formulated in nutraceutical and pharmaceutical products. The demand is met by concentrating these PUFAs from alga, fish oil, fish by-products and edible oil (Chakraborty *et al.*, 2010). Purification and concentration of PUFAs can be best done using lipases and many bacterial lipases have been studied for their efficiency in enhancing PUFA content (Kumar *et al.*, 2005; Chakraborty and Paulraj, 2008).

Kojima *et al.* (2006) used two lipases (AK-lipase and HU-lipase) produced by *P. fluorescens* to selectively concentrate EPA and DHA from fish oils. This method is economical for concentrating PUFAs when compared to the methods which involve distillation, chromatography and fluid extraction which are cumbersome and costly. The hydrolysis of cuttle-fish oil using AK-lipase enhanced the DHA composition from 16.3 to 44.6%. The composition of EPA in cod-oil was increased from 12 to 43% using HU-lipase. Byun *et al.* (2007) have used a *Pseudomonas* lipase to hydrolyse sardine oil in the presence of emulsifiers and observed a decrease in the level of saturated fatty acids and increase in the levels of mono and poly unsaturated fatty acids after hydrolysis. Yamauchi *et al.* (2005) have purified arachidonic acid from *Mortierella alpine* single-cell oil using lipases from *Alcaligenes* and *B. cepacia*. The process involved a nonselective hydrolysis of the oil using *Alcaligenes* lipase followed by a selective elimination of saturated fatty acids using urea adduct fractionation and finally a selective enrichment by esterification using *B. cepacia* lipase.

Modification of fat and oil: The health potential of fats and oils depends largely on the distribution pattern of the fatty acid present in them. Tailored vegetable oils with structured triglycerides popularly known as structured lipids are desirable as they contribute health benefits. Many vegetable oils like sunflower, coconut, olive, corn and rice bran oil are rich in ω6 fatty acids and fish, linseed oil, walnuts and milk are rich in ω3 fatty acids. Though both these poly unsaturated fatty acids play a predominant role in contributing to the health of an individual, the

ingested ratio of ω6/ω3 needs to be monitored. This ratio is significant and should be balanced between 1 and 4; a high ω6/ω3 ratio may pose health hazards (Griffin, 2008). The use of vegetable oils particularly those enriched with ω6 fatty acids results in high ω6/ω3 ratio in the diet. The best strategy to improve the ω6/ω3 ratio is the enzymatic modification method which uses lipase-catalysed interesterification (Mitra *et al*., 2010). Dioleyl palmitoyl glycerol (OPO) is a structured triglyceride produced by acidolysis of tripalmitin with oleic acid. Guncheva *et al*. (2008) evaluated the efficiency of a lipase from *B. stearothermophilus* MC7 in producing OPO. Synthesis of a structured lipid requires high temperatures for better homogenization during the process and presence of organic solvents to assist the lipase to resist high temperatures. The presence of organic solvents may sometimes prove to be disadvantageous as undesired isomeric products form. Lipase MC7 could operate in non-solvent high temperature reaction systems and thus averted the side reactions of acidolysis.

Glycerolysis: Monoacyl Glycerols (MAGs) and Diacyl Glycerols (DAGs) are surface-active molecules that are widely used as emulsifiers in food, pharmaceutical and personal-care products. They possess excellent emulsifying properties and are traditionally produced by chemical glycerolysis. DAGs have also gained attention due to their positive impact on health unlike triglycerides (Cheirsilp *et al*., 2009; Kahveci *et al*., 2009). Enzymatic glycerolysis averts the negative side effects of chemical process like products with undesirable color and flavor (Cheirsilp *et al*., 2009). There are several studies on lipase-catalyzed glycerolysis (Guo and Xu, 2005; Kristensen *et al*., 2005).

Synthesis of flavor esters: Flavour compounds that are extracted from plants are too expensive and hence are replaced by flavor esters synthesized using catalysts. Flavor or fragrance materials which include various aliphatic and aromatic compounds share a major market of food additives throughout the world. Flavor esters are low molecular weight compounds synthesized by the esterification of fatty acids, preferably by microbial lipases. These compounds carry the tag natural, despite being synthesized and hence are a subject of intensive research (Gillies *et al*., 1987). Some of the esters synthesized by esterification reactions catalysed by bacterial lipases are ethyl acetate, ethyl butyrate, ethyl methyl butyrate, ethyl valerate and ethyl caprylate (Talon *et al*., 1996; Dandavate *et al*., 2009; Ahmed *et al*., 2010).

Tea seed oil: Cocoa butter is a primary ingredient of dark chocolates and the high price of cocoa and cocoa butter prompts to find an alternative cheaper source. Interesterified tea seed oil was identified as a best replacement for cocoa butter and chocolates made with tea seed oil resembled those made with cocoa butter. Tea seed oil is a by-product of tea processing and thus partial substitution of coca butter reduces the cost of confectionary products (Zarringhalami *et al*., 2010).

Lipolysed milk fat (LMF): The LMF is prepared from condensed milk or butter oil using lipases which release free fatty acids and give it a cheesy aroma. LMF is used in chocolate coatings, artificial flavor additives, margarine etc. The bacterial lipases used to prepare LMF include those obtained from *Achromobacter* and *Pseudomonas* sp.

Cheese: Lipases from *Lactobacillus* play a prominent role in manufacture of bacterial ripened cheese like Parmesan and Grana Padano cheese. Mandrich *et al*. (2006) have investigated the role

of lipase/esterase from *Alicyclobacillus acidocaldarius* in milk and cheese models. They observed that the recombinant enzyme was more efficient than the native enzyme and could be used in dairy industry to impart flavor or enhance cheese ripening.

Enzyme Modified Cheese (EMC) is a concentrated cheese flavor food ingredient produced by treating cheese curd with enzymes. It is used as cheese powders, in soups, salads, sauces and coatings (Guinee and Kilcawley, 2004). EMC has more intense flavor than naturally ripened cheese and small chain fatty acids (C2-C6) contributes primarily to this flavor (www.amano-enzyme.co.jp). Esterification produces new esters like ethyl butanoate, ethyl hexanoate which confer characteristic flavors to cheese (Fenster *et al*., 2003).

Bread making: Emulsifiers are additives required as a bread improver. Bread improvers improve bread volume and texture and dough stability. These emulsifiers are detectable in the final baked and marketed loaf and thus find a place in the label. Enzymes on the other hand get denatured during baking and thus provide bread improving functions without appearing on the label. Lipase from *B. subtilis* has been proved to play a role in bread making in a study by Sanchez *et al*. (2002). These enzymes can completely or partially replace the traditional volume improving agents (Moayedallaie *et al*., 2010).

Detergents: The most noteworthy application of hydrolytic lipases is their use in house-hold and laundry detergents. Lipases were developed as detergent enzymes after the successful introduction of proteases in powder and liquid detergents. Lipases should meet the following criteria to serve as detergent additives: stability at alkaline pH, solubility in water, tolerance to detergent proteases and surfactants and low substrate specificity (Quax, 2006; Rahman *et al*., 2006). Genencor International introduced commercial bacterial lipases Lipomax from *P. alcaligenes* and Lumafast from *P. mendocina* which could be used as detergent enzymes in the year 1995 (Rahman *et al*., 2006). Though fungal lipase was the first introduced detergent enzyme, bacterial lipases captivated the detergent market. This was due to the acidophilic nature of fungal enzymes that make them incompatible with the alkaline wash conditions (Quax, 2006). During laundering, the lipase adsorbs on to the fabric surface to form a stable fabric-lipase complex which then acts on the oil stains and hydrolyses them. The complex is resistant to the harsh wash conditions and is retained on the fabric during laundering (Hasan *et al*., 2006).

A detergent stable lipase was isolated from *B. cepacia* by Rathi *et al*. (2001). The enzyme was found to meet all the criteria necessary for a detergent additive and exhibited better stability than Lipolase, a detergent stable lipase marketed by Novo Nordisk, Denmark. Suzuki (2001) has patented an alkaline *Pseudomonas* lipase which remains active at low temperatures and has improved wash performance. The patent also pertains to a detergent composition containing such lipase. Wang *et al*. (2009) and Zhang *et al*. (2009a, b) have isolated lipases from *B. cepacia* and *P. fluorescens* and the lipases proved to be suitable for detergent industry. A lipase isolated from *B. licheniformis* was not stable and lost its activity in the presence of commercial detergents but its activity was restored by the addition of calcium chloride to the enzyme-detergent complex (Bayoumi *et al*., 2007). Such lipases lose their activities in the presence of a chelating agent, if any, in the detergent.

Tannery: Leather processing operations are classified in to three groups: pre-tanning which cleans the hides or skins, tanning which stabilizes them and finally post-tanning which adds aesthetic

value to it (Thanikaivelan *et al*., 2004). All these stages use many chemicals and enzymes. Enzymatic leather processing was at the research level in the early 19th century and only in the year 1980 did industrial scale operations were done. The different stages of leather processing are curing, soaking, liming, dehairing, bating, pickling, degreasing and tanning. Lipases are employed in soaking, bating and degreasing stages (Choudary *et al*., 2004). Soaking is the first stage of leather processing and is performed to rehydrate the skin or hide. Conventional processes use soda ash or sodium tetrasulphide in the presence of surfactant while enzymatic processes use lipase along with protease Tancous *et al*. (1994) cited in Choudary *et al*. (2004). Forezym SK is a commercial bioproduct comprising of a mixture of bacterial protease and lipase marketed La Forestal Tanica, Spain. Dehairing is a process of removing hair from skin and hides. Loosening of the hair is performed by lime and sulphide in conventional processes while enzymatic processes depend on proteases. Dorezym LM is also a mixture of bacterial protease and lipase marketed by La Forestal Tanica despite the role of lipase in dehairing being unclear. Degreasing is performed to the remove the residual grease left out after liming process. Large quantities of natural fat present in the skin, particularly sheep skin cannot be removed by the liming operation and hence requires a subsequent degreasing process. Degreasing initially performed with lipase alone was not satisfactory and hence a combination of protease and lipase was evaluated. The degreasing process involves breaking down of the protein membrane of the fat sac, removal of the fat and its emulsification in water or solvent. Hence of a combination of protease and lipase and an enzyme-compatible surfactant becomes necessary for degreasing effect (Thanikaivelan *et al*., 2004). Both acid and neutral lipase has been used for degreasing. La Forestal Tanica has marketed Forezym WG-L, Forezym DG as degreasing bacterial lipases.

Textile industry: Denim culture has spread all over the world and India has witnessed alluring changes in denim fashion. Garment processing promises a refined and polished look to the finished fabric. Desizing is such a process and is required to remove the size material which has been impregnated from the fabric prior to weaving. Traditional desizing uses acid or oxidizing agents which damages the cellulose material in the fabric. Enzymatic desizing has multiple advantages over the traditional process and uses enzymes like cellulase, amylase, protease and lipase depending on the sizing agent (www.expresstextile.com). Bacterial lipases can be used if the size material is a synthetic sizing agent like polyesters (www.wipo.int). Lund (2001) has patented a process for combined desizing and stone-washing of denim. Lipolytic enzymes from *P. cepacia*, *P. fluoresecens*, *P. fragi* and *P. stutzeri* were evaluated and the lipase of *P. cepacia* was preferred.

Polyethylene Terephthalate (PET) fabrics are known for their strength and wrinkle resistance. The lack of dyeability is its undesirable feature which is because of its hydrophobic character. Any attempt to increase its moisture regaining nature will make the PET fabrics more fashionable. Kim and Song (2006) have used lipases from *P. cepacia* and *P. fluorescens* in an eco-friendly method to improve the moisture regain of PET fabrics and found it to be more effective than the alkaline method.

Synthesis of polymers: Polylactate (PLA) fibres are made of lactic acid obtained by fermentation of natural sugars. It is an eco-friendly material which has low energy consumption and high bio-degradability. It is used in textile fabrics, packaging material, bio-medicine and as bio-plastics (Drumright *et al*., 2000). PLA fibre is hydrophobic and thus the affinity of dyes and chemicals to it is less. This necessitates the modification of the surface of the fibres which makes them more

wettable. Since ester bonds are present in poly-lactate, the surface can be modified enzymatically using lipase (Wang *et al*., 2002).

PLA has mechanical properties similar to those of polystyrene and polyethylene tetraphthalate and can be obtained from different isomers of lactic acid (Garcia-Arrazola *et al*., 2009). Poly-L-Lactic Acid (PLLA) is semi-crystalline in nature and is more potential than amorphous PLA (Lim *et al*., 2008). The enzymatic synthesis of PLLA using lipase from *B. cepacia* has been reported (Matsumura *et al*., 1997).

Pharmaceutical applications: Miyazaki and Fujikawa (2009) have patented methods to treat skin, scalp disease and hair-loss using compositions that contain a protease, lipase and a metallic salt. The lipase could be obtained from a fungal or bacterial source, preferably *Pseudomonas*. Sani (2006) has proposed that bacterial lipases can replace pancreatic lipases used to treat cystic fibrosis and pancreatitis. Pancreatic lipase is used as a digestive aid in lipid malabsorption disorders but its application is limited as it loses its efficacy at low pH and in the presence of protease. Many bacterial lipases are hence promising alternatives to pancreatic lipase.

Phenolics are natural antioxidants which lose their activity in stabilizing oils. This disadvantage is due to their hydrophilic nature. Solubility of phenolics in a fatty region becomes necessary for it to exhibit its action and thus production of lipophilic phenols will make the complete usage of the antioxidant properties of phenols feasible (Choo and Birch, 2009). Biocatalytic synthesis of cinnamoyl esters using *Pseudomonas* lipase has been reported (Buisman *et al*., 1998) and cinnamoyl esters have higher free radical scavenging activity than cinnamic acid.

An enantioselective lipase from *Acinetobacter* hydrolysed cis-(±)-2-(bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methyl acetate. This is a racemic intermediate in the synthesis of Itraconazole, an antifungal agent (Han *et al*., 2003). Diltiazem is a calcim channel blocker and is used to treat angina pectoris, hypertension and other vascular disorders. Synthesis of Diltiazem hydrochloride requires an optically pure (-) trans-methoxylphenyl glycidic acid methyl ester (Zhao *et al*., 2008). A lipase produced from *Serratia marescens* was efficient to catalyse the bioresolution of (±) MPGM (Hu *et al*., 2009). A Serratia marescens lipase was used for the bioresolution of racemic ketoprofen, a non-steroidal anti-inflammatory drug (Long *et al*., 2007).

Hydrocinnamate esters are precursors for the synthesis of 1,3,4,9-tetrahydropyrano [3,4-b]indole-1-acetic acid which is used as a analgesic, antipyretic and anti-inflammatory agent. These esters also act as inhibitors of HIV-protease. These esters have been synthesized using *P. cepacia* lipase by Priya and Chadha (2003).

Biosensors: Qualitative and quantitative determination of lipids and lipid-binding proteins is possible with the help of biosensors which may be of chemical or biochemical in nature. Bacterial lipases have been used as biosensors and this exploits immobilized lipases. Such biosensors are used to detect triglycerides in food and clinical samples, pollution analysis like pesticide contamination and pharmaceutical industry (Pandey *et al*., 1999). Huang *et al*. (2001) immobilized lipase on a micro-emulsion based gel to fabricate a glass-electrode-based lipase biosensor. Setzu *et al*. (2007) fabricated a potentiometric biosensor using a lipase immobilized on a mesoporous silica matrix.

Agrochemical industry: Lipases are used to synthesise intermediates involved in the production of pesticides, insecticides and other agrochemically useful compounds. Transesterification and resolution by *Pseudomonas* lipases was studied to produce insecticides, fungicides (Pandey *et al*.,

1999) and secondary alcohols like (R, S)-HMPC [(R, S)-4-hydroxy-3-methyl-2-(2'-propenyl)-2-cyclopenten-1-one] (Xu *et al*., 2005).

Cosmetics and personal care products: Production of flavors by transesterification and resolution of racemic intermediates by lipases boosts the cosmetic and perfume industry. Lipases produced by *P. cepacia* have been used to resolve the racemic rose oxides produced by the bromomethoxylation of citronellol (Taneja *et al*., 2005).

Esters of aliphatic and aromatic acids, alcohols including terpene alcohols, aldehydes, phenols are commonly present in the flavor materials used in perfumes and other personal care products (Franssen *et al*., 2005). Priya and Chadha (2003) studied the synthesis of hydrocinnamic acid esters by *P. cepacia*. The esters of hydrocinnamic acid are used in perfumes and sunscreens. Mouth washes and shaving creams contain menthol to provide a peppermint flavor and a cooling sensation. Menthol can be artificially produced by the esterification process when there is a dearth for the natural menthol. Chaplin *et al*. (2006) have patented a process to produce menthol esters and similar compounds and this process utilizes lipases of *P. fluorescens* and *P. cepacia*.

Biodiesel production: Biodiesel is an alternative fuel for petroleum-based diesel and is biodegradable, renewable, non-inflammable and non-toxic. Biodiesel is defined as monoalkyl esters of long chain fatty acids usually a methyl ester of fatty acid and denoted commonly as FAME (fatty acid methyl ester). Biodiesel is used for diesel engines and heating systems and its demand has increased due to the soaring petroleum prices. Biodiesel is produced from vegetable oils such as soybean oil, rice bran oil, sunflower oil, palm oil, cotton seed oil and jatropha oil, animal fat, algae, waste edible oil and industrial acid oil.

Biodiesel is synthesized by chemocatalytic, thermocatalytic and biocatalytic approaches where, the latter employs lipases as biocatalysts. The lipase catlysed transesterification reaction takes place between a lipid and a short chain alcohol to produce an ester and glycerol (Chen *et al*., 2009; Dizge *et al*., 2009; Raita *et al*., 2010). The most commonly employed bacterial lipase for biodiesel synthesis is from *P. cepacia* (Li and Yan, 2008). The production of biodiesel requires a micro-aqueous environment because the presence of water does not promote transesterification rather favours the hydrolysis of oil. Hence immobilized lipases are employed to function in a micro-aqueous of solvent-free system (Xu *et al*., 2009). Wang and Zhang (2009) studied the presence of compatible solutes to improve methanolysis for biodiesel production. Methanol competitively inhibits lipase and also denatures it and thus decreases biodiesel production. A solute like ectoine decreases the affinity of the lipase for methanol but increases its affinity for the triglyceride and this strategy improved the yield of biodiesel.

Glycerol, the by-product of biodiesel production is converted to 1,3 propanediol which is a monomer for the synthesis of novel polymers like polymethylene terephthalate. Bacterial species like *Klebsiella, Citrobacter, Clostridium, Enterobacter* and *Lactobacillus* can convert glycerol to 1, 3 propanediol (Xu *et al*., 2009).

Environment management: Waste-water treatment processes remove lipidic residues by air-floatation and discard it in sanitary landfill dumping yard. These pose a threat to the waterbeds and ground water by decreasing the oxygen transfer rate and hence bio-remediation is adopted to control pollution (Mongkolthanaruk and Dharmsthiti, 2002). Oil spills in the soil and water during rigging and refining can be handled using lipases (Pandey *et al*., 1999). The most common method

of treating waste water is by cultivating pure cultures which produce lipase and mixed cultures which produce lipase and other enzymes. Effluents emanating from food processing, tannery, automobile industries and restaurant and fast-food outlets can be treated with by cultivating lipase producing bacteria (Pandey *et al*., 1999; Nelson and Rawson, 2010). The commonly used bacterial genera are *Pseudomonas*, *Bacillus* and *Acinetobacter* (Mongkolthanaruk and Dharmsthiti, 2002).

Environmental studies: Biogeochemical studies include the analysis of the relationship between Dissolved Organic Matter (DOM) and bacterial dynamics, the response of bacteria to environmental conditions etc. These studies utilize various parameters for analysis and bacterial activities are one among them. Bacteria use the readily available pool of DOM for their growth and breakdown major molecules enzymatically when the small molecules deplete. Thus the analysis of bacterial enzyme activities like amino peptide hydrolysis and polysaccharide degradation *in situ* was studied as indicators of DOM dynamics. Recently Bourguet *et al*. (2009) studied the relationship between the levels of lipids and *in situ* lipase activities during different environmental conditions like Spring and Summer.

CONCLUDING REMARKS

Despite enzymes of fungal origin holding a major share in the global market for lipases, bacterial lipases have been extensively studied and their commercialization has improved. The knowledge of the potential of bacterial lipases has increased and these enzymes have been employed by various industrial sectors successfully. Lipases are inducible and their yield has been improved using recombinant DNA technology. They are effective biocatalysts whose efficiency has been improved by immobilization and bioimprinting. The applications of bacterial lipases in food, textiles, detergents, pharmaceutical and tannery have been well explored.

REFERENCES

Aguilar, C.N., E.F. Torres, G.V. Gonzalez and C. Augur, 2002. Culture conditions dictate protease and tannase production in submerged and solid-state cultures of *Aspergillus niger* Aa-20. Appl. Biochem. Biotechnol., 102: 407-414.

Ahmed, E.H., T. Raghavendra and A.D. Madamwar, 2009. Thermostable alkaline lipase from a local isolate *Bacillus subtilis* EH 37: Characterization, partial purification and application in organic synthesis. Appl. Biochem. Biotechnol., 160: 2102-2113.

Ahmed, E.H., T. Raghavendra and D. Madamwar, 2010. An alkaline lipase from organic solvent tolerant *Acinetobacter* sp. EH28: Application for ethyl caprylate synthesis. Bioresour. Technol., 101: 3628-3634.

Akbari, N., K. Khajeh, S. Rezaie, S. Mirdamadi, M. Shavandi and N. Ghaemi, 2010. High-level expression of lipase in *Escherichia coli* and recovery of active recombinant enzyme through *in vitro* refolding. Protein Expr. Purif., 70: 75-80.

Al-Zuhair, S., A. Dowaidar and H. Kamal, 2009. Dynamic modeling of biodiesel production from simulated waste cooking oil using immobilized lipase. Biochem. Eng. J., 44: 256-262.

Alkan H, Z. Baysal, F. Uyar and M. Dogru, 2007. Production of lipase by a newly isolated *Bacillus coagulans* under solid-state fermentation using melon wastes. Appl. Biochem. Biotechnol., 136: 183-192.

An, S.Y., S.W. Kim, Y.L. Choi, Y.S. Cho, W.H. Joo and Y.C. Lee, 2003. Cloning, expression in *Escherichia coli* and enzymatic properties of a lipase from *Pseudomonas* sp.SW-3. J. Microbiol., 41: 95-101.

Angkawidjaja, C. and S. Kanaya, 2006. Family I.3 lipase: Bacterial lipases secreted by the type I secretion system. Cell. Mol. Life Sci., 63: 2804-2817.

Arpigny, J.L. and K.E. Jaeger, 1999. Bacterial lipolytic enzymes: Classification and properties. Biochem. J., 343: 177-186.

Babu, I.S. and G.H. Rao, 2007. Optimization of process parameters for the production of lipase in submerged fermentation by *Yarrowia lipolytica* NCIM 3589. Res. J. Microbiol., 2: 88-93.

Bayoumi, R.A., S.S. El-louboudey, N.M. Sidkey and M.A. Abd-El-Rahman, 2007. Production, purification and characterization of thermoalkalophilic lipase for application in bio-detergent industry. J. Appl.Sci. Res., 3: 1752-1765.

Boekema, A.B., M. Breuer, B. Hauer, M. Koster, F. Rosenau, K.E. Jaeger and J. Tommassen, 2007. Hexadecane and tween 80 stimulate lipase production in *Burkholderia glumae* by different mechanisms. Appl. Env. Microbiol., 73: 3838-3844.

Bourguet, N., M. Goutx, J-F. Ghiglione, M. Pujo-Pay and G. Mevel *et al*., 2009. Lipid biomarkers and bacterial lipase activities as indicators of organic matter and bacterial dynamics in contrasted regimes at the DYFAMED site, NW Mediterranean. Deep Sea Res., 56: 1454-1469.

Buisman, G.J.H, C.T.W. van Helteren, G.F.H. Kramer, J.W. Veldsink, J.T.P. Derksen and F.P. Cuperus, 1998. Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol. Lett., 20: 131-136.

Buist, G., A.N.J.A. Ridder, J. Kok and O.P. Kuipers, 2006. Different subcellular locations of secretome components of Gram-positive bacteria. Microbiol., 152: 2867-2874.

Byun, H.G., T.K. Eom, W.K. Jung and S.K. Kim, 2007. Lipase catalysed hydrolysis of fish oil in an optimum emulsion system. Biotechnol. Bioprocess Eng., 12: 484-490.

Cadirci, B.H. and I. Yasa, 2009. An organic solvents tolerant and thermotolerant lipase from *Pseudomonas fluorescens* P21. J. Mol. Catal. B. Enz., 64: 155-167.

Cai, Y., L. Wang, X. Liao, Y. Ding and J. Sun, 2009. Purification and partial characterization of two new cold-adapted lipases from mesophilic *Geotrichum* sp. SYBC WU-3. Process Biochem., 44: 786-790.

Cao, X., J.Yang, L. Shu, B.Yu and Y. Yan, 2009. A Improving esterification activity of *Burkholderia cepacia* lipase encapsulated in silica by bioimprinting with substrate analogues. Process Biochem., 44: 177-182.

Chakraborty, K. and R. Paulraj, 2008. An extra-cellular alkaline metallolipase from *Bacillus licheniformis* MTCC 6824: Purification and biochemical characterization. Food Chem., 109: 727-736.

Chakraborty, K., P. Vijayagopal, R.D. Chakraborty and K.K. Vijayan, 2010. Preparation of eicosapentaenoic acid concentrates from sardine oil by *Bacillus circulans* lipase. Food Chem., 120: 433-442.

Chaplin, J.A., N. Gardiner, R.K. Mitra, C.J. Parkinson and M. Portwig *et al*., 2006. Process for preparing (-) menthol and similar compounds. United States Patent 7026144. http://www.freepatentsonline.com

Cheirsilp, B., P. Jeamjounkhaw and A.H. Kittikun, 2009. Optimizing an alginate immobilized lipase for monoacylglycerol production by the glycerolysis reaction. J. Mol. Catal. B. Enz., 59: 206-211.

Chen, Y., B. Xiao, J. Chang, Y. Fu, P. Lv and X. Wang, 2009. Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor. Energy Convers. Manage., 50: 668-673.

Choo, W.S. and E.J. Birch, 2009. Radical scavenging activity of lipophilized products from lipase-catalyzed transesterification of triolein with cinnamic and ferulic acids. Lipids, 44: 145-152.

Choudary, R.B., A.K. Jana and M.K. Jha, 2004. Enzyme technology applications in enzyme processing. Indian J. Chem. Tech., 11: 659-671.

Dandavate, V., J. Jinjala, H. Keharia and D. Madamwar, 2009. Production, partial purification and characterization of organic solvent tolerant lipase from *Burkholderia multivorans* V2 and its application for ester synthesis. Bioresour. Technol., 100: 3374-3381.

De Pascale, D., A.M. Cusano, F. Autore, E. Parrilli, G. Di Prisco, G. Marino and M.L. Tutino, 2008. The cold-active Lip1 lipase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 is a member of a new bacterial lipolytic enzyme family. Extremophiles, 12: 311-323.

Devi, B.L.A.P., Z. Guo and X. Xu, 2009. Characterization of cross-linked lipase aggregates. J. Am. Oil Chem. Soc., 86: 637-642.

Dizge, N., C. Aydiner, D.Y. Imer, M. Bayramoglu, A. Tanriseven and B. Keskinler, 2009. Biodiesel production from sunflower, soybean and waste cooking oils by transesterification using lipase immobilized onto a novel microporous polymer. Bioresour. Technol., 100: 1983-1991.

Drumright, R.E., P.R. Gruber and D.E. Henton, 2000. Polylactic acid technology. Adv. Mater., 12: 1841-1846.

Dutta, S. and L. Ray, 2009. Production and characterization of an alkaline thermostable crude lipase from an isolated strain of *Bacillus cereus* C7. Appl. Biochem. Biotechnol., 159: 142-154.

Ertugrul, S., G. Donmez and S. Takac, 2007. Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. J. Haz. Mater., 149: 720-724.

Fenster, K.M., S.A. Rankin and J.L. Steele, 2003. Accumulation of short n-chain ethyl esters by esterases of lactic acid bacteria under conditions simulating ripening parmesan cheese. J. Dairy Sci., 86: 2818-2825.

Fickers, P., M. Ongena, J. Destain, F. Weekers and P. Thonart, 2006. Production and down-stream processing of an extracellular lipase from the yeasr *Yarrowia lipolytica*. Enzyme Microb. Technol., 38: 756-759.

Franssen, M.C.R., L. Alessandrini and G. Terraneo, 2005. Biocatalytic production of flavors and fragrances. Pure Appl. Chem., 77: 273-279.

Furukawa, S., T. Ono, H. Ijima and K. Kawakami, 2002. Effect of imprinting sol-gel immobilized lipase with chiral template substrates in esterification of (R)-(+)-and (S)-(-)-glycidol. J. Mol. Catal. B. Enz., 17: 23-28.

Garcia-Arrazola, R., A. David, L. Guerrero, M. Gimeno and E. Barzana, 2009. Lipase-catalyzed synthesis of poly-l-lactide using supercritical carbon dioxide. J. Supercritical Fluids., 51: 197-201.

Ghazali, H.M., S. Hamidah and Y.B. Che Man, 1995. Enzymatic transesterification of palm olein with nonspecific and 1,3-specific lipases. J. Am. Oil Chem. Soc., 72: 633-639.

Gilham, D. and R. Lehner, 2005. Techniques to measure lipase and esterase activity *in vitro*. Methods, 36: 139-147.

Gillies, B., H. Yamazaki and D.W. Armstrong, 1987. Production of flavor esters by immobilized lipase. Biotechnol. Lett., 7: 709-714.

Goldberg, A.L., 2003. Protein degradation and protection against misfolded or damaged proteins. Nat., 426: 895-899.

Griffin, B.A., 2008. How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. Curr. Opin. Lipidol., 19: 57-62.

Guinee, T.P. and K.N. Kilcawley, 2004. Cheese as an Ingredient. In: Cheese: Chemistry, Physics and Microbiology, Fox, P.F., P.L.H. McSweeney, T.M. Cogan and T.P. Guinee (Eds.). Elsevier Academic Press, Amsterdam.

Gunasekaran, G., S.M. Kotay and D. Das, 2006. Alkaline lipase production by citrobacter freundii IIT-BT L139. Indian J. Exp. Biol., 44: 485-491.

Guncheva, M., D. Zhiryakova, N. Radchenkova and M. Kambourova, 2008. Acidolysis of tripalmitin with oleic acid catalyzed by a newly isolated thermostable lipase. J. Am. Oil Chem. Soc., 85: 129-132.

Guncheva, M., D. Zhiryakova, N. Radchenkova and M. Kambourova, 2009. Properties of immobilized lipase from *Bacillus stearothermophilus* MC7. Acidolysis of triolein with caprylic acid. World J. Microbiol. Biotechnol., 25: 727-731.

Guo, Z. and X. Xu, 2005. New opportunity for enzymatic modification of fats and oils with industrial potentials. Org. Biomol. Chem., 3: 2615-2619.

Han, S., J.H. Back, M.Y. Yoon, P.K. Shin and C.S. Cheong *et al*., 2003. Expression and characterization of a novel enantioselective lipase from *Acinetobacter* species SY-01. Biochem., 85: 501-510.

Hartmann, M., 2005. Ordered mesoporous materials for bioadsorption and biocatalysis. Chem. Mater., 17: 4577-4593.

Hasan, F., A.A. Shah and A. Hameed, 2006. Industrial applications of microbial lipases. Enzyme Microb. Technol., 39: 235-251.

Hasan, F., A.A. Shah and A. Hameed, 2009. Methods for detection and characterization of lipases: A comprehensive review. Biotechnol. Adv., 27: 782-798.

He, P., G. Greenway and S.J. Haswell, 2010. Development of a monolith based immobilized lipase micro-reactor for biocatalytic reactions in a biphasic mobile system. Process Biochem., 45: 593-597.

Hu, B., J. Pan, H.L. Yu, J.W. Liu and J.H Xu, 2009. Immobilization of *Serratia marcescens* lipase onto amino-functionalized magnetic nanoparticles for repeated use in enzymatic synthesis of diltiazem intermediate. Process Biochem., 44: 1019-1024.

Huang, X.R., Y.Z. Li, G.L. Yang, L.L. Liu, Y.B. Qu and W.J. Zhang, 2001. A novel method for fabrication of a glass-electrode-based lipase sensor. Chinese Chem. Lett., 12: 453-456.

Hun, C.J., R.N.Z.A. Rahman, A.B. Salleh and M. Basri, 2003. A newly isolated organic solvent tolerant *Bacillus sphaericus* 205y producing organic solvent-stable lipase. Biochem. Eng. J., 15: 147-151.

Jaladi, H., A. Katiyar, S.W. Thiel, V.V. Guliants and N.G. Pinto, 2009. Effect of pore diffusional resistance on biocatalytic activity of *Burkholderia cepacia* lipase immobilized on SBA-15 hosts. Chem. Eng. Sci., 64: 1474-1779.

Joseph, B., P.W. Ramteke and G. Thomas, 2008. Cold active microbial lipases: Some hot issues and recent developments. Biotechnol. Adv., 26: 457-470.

Kahveci, D., Z. Guo, B. Ozcelik and X. Xu, 2009. Lipase-catalyzed glycerolysis in ionic liquids directed towards diglyceride synthesis. Process Biochem. 44: 1358-1365.

Kang, H., J. Kim, M. Kim, S. Park, T. Oh and C. Hur, 2009. MELDB: A database for microbial esterases and lipases. FEBS Lett., 580: 2736-2740.

Kato, K. and S. Seelan, 2010. Enhancing activity and stability of *Burkholderia cepacia* lipase by immobilization on surface-functionalized mesoporous silicates. J. Biosci. Bioeng., 109: 615-617.

Kim, E.K., W.H. Jang, J.H. Ko, J.S. Kang, M.J. Noh and O.J. Yoo, 2001. Lipase and its modulator from *Pseudomonas* sp. strain KFCC 10818: Proline-to-glutamine substitution at position 112 induces formation of enzymatically active lipase in the absence of the modulator. J. Bacteriol., 183: 5937-5941.

Kim, H.K., H.J. Choi, M.H. Kim and T.K. Oh, 2002. Expression and characterization of Ca^{2+}-independent lipase from *Bacillus pumilus* B26. Biochim. Biophys. Acta., 1583: 205-212.

Kim, H.R. and W.S. Song, 2006. Lipase treatment of polyester fabrics. Fibers Polym., 7: 339-343.

Kojima, Y., E. Sakuradani and S. Shimizu, 2006. Different specificities of two types of *Pseudomonas* lipases for C20 fatty acids with a Δ5 unsaturated double and their application for selective concentration of fatty acids. J. Biosci. Bioeng., 1: 490-500.

Kristensen, J.B., X. Xu and H. Mu, 2005. Process optimization using response surface design and pilot plant production of dietary diacylglycerols by lipase-catalyzed glycerolysis. J. Agric. Food Chem., 53: 7059-7066.

Kumar, S., K. Kikon, A. Upadhyay, S.S. Kanwar and R. Gupta, 2005. Production, purification and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. Protein Expr. Purif., 41: 38-44.

Lee, D.W., H.W. Kim, K.W. Lee, B.C. Kim and E.A. Choe *et al*., 2001. Purification and characterization of two distinct thermostable lipases from the gram-positive thermophilic bacterium *Bacillus thermoleovorans* ID-1. Enzyme Microb. Technol., 29: 363-371.

Lee, S.H. and D.H. Park, 2008. Isolation and physiological characterization of *Bacillus clausii* SKAL-16 isolated from wastewater. J. Microbiol. Biotechnol., 18: 1908-1914.

Lee, D.G., K.M. Ponvel, M. Kim, S. Hwang, I.S. Ahn and C.H. Lee, 2009. Immobilization of lipase on hydrophobic nano-sized magnetite particles. J. Mol. Catal. B. Enz., 57: 62-66.

Lee, H.K., J.K. Lee, M.J. Kim and C.J. Lee, 2010. Immobilization of lipase on single walled carbon nanotubes in ionic liquid. Bull. Korean Chem. Soc., 31: 650-652.

Li, C.Y., C.Y. Cheng and T.L. Chen, 2004. Fed-batch production of lipase by *Acinetobacter radioresistens* using Tween 80 as the carbon source. Biochem. Eng. J., 19: 25-31.

Li, Q. and Y. Yan, 2008. Production of biodiesel catalyzed by immobilized *Pseudomonas* cepacia lipase from *Sapium sebiferum* oil in micro-aqueous phase. Appl. Energ., 10.1016/j.apenergy.2010.02.032

Lim, L.T., R. Auras and M. Rubino, 2008. Processing technologies for poly(lactic acid). Prog. Polym. Sci., 33: 820-852.

Lianghua, T. and X. Liming, 2005. Purification and partial characterization of a lipase from *Bacillus coagulans* ZJU318. Applied Biochem. Biotechnol., 125: 139-146.

Liu, C.H., Y.H. Lin, C.Y. Chen and J.S. Chang, 2009. Characterization of *Burkholderia* lipase immobilized on celite carriers. J. Taiwan Inst. Chem. Eng., 40: 359-363.

Long, Z.D., J.H. Xu, L.L. Zhao, J. Pan, S. Yang and L. Hua, 2007. Overexpression of *Serratia marcescens* lipase in *Escherichia coli* for efficient bioresolution of racemic ketoprofen. J. Mol. Catal. B. Enzym., 27: 105-110.

Lopes, M., N. Gomes, C. Goncalves, M.A.Z. Coelho, M. Mota and I. Belo, 2008. *Yarrowia lipolytica* lipase production enhanced by increased air pressure. Lett. Appl. Microbiol., 46: 255-260.

Lund, H., 2001. Process for combined desizing and stone-washing of dyed denim. United States Patent 6261828. http://www.freepatentsonline.com

Madan, B. and P. Mishra, 2010. Co-expression of the lipase and foldase of *Pseudomonas aeruginosa* to a functional lipase in *Escherichia coli*. Appl. Microbiol. Biotechnol., 85: 597-604.

Mandrich, L., G. Manco, M. Rossi, E. Floris, T.J. van den Bosch, G. Smit and J.A. Wouters, 2006. *Alicyclobacillus acidocaldarius* thermophilic esterase EST2's activity in milk and cheese models. Appl. Env. Microbiol., 72: 3191-3197.

Matsumura, S., K. Mabuchi and K. Toshima, 1997. Lipase-catalyzed polymerization of Lactide. Macromol. Rapid. Commun., 18: 477-482.

Mingarro, I., C. Abad and L. Braco, 1995. Interfacial activation-based molecular bioimprinting of lipolytic enzymes. Proc. Natl. Acad. Sci., 92: 3308-3312.

Minovska, V., E. Winkelhausen and S. Kuzmanova, 2005. Lipase immobilized by different techniques on various support materials applied in oil hydrolysis. J. Serb. Chem. Soc., 70: 609-624.

Mitra, K., S.A. Kim, J.H. Lee, S.W. Choi and K.T. Lee, 2010. Production and characterization of α-linolenic acid enriched structured lipids from lipase-catalyzed interesterification. Food Sci. Biotechnol., 19: 57-62.

Miyazaki, K. and S. Fujikawa, 2009. Methods of treating skin disease, scalp disease, sensitive skin or suppressing hair loss with microbubble washing compositions. US patent number 200090214512. http://www.freepatentsonline.com

Mnisi, S.M., M.E. Louw and J. Theron, 2005. Cloning and characterization of a carboxylesterase from *Bacillus coagulans* 81-11. Curr. Microbiol., 50: 196-201.

Moayedallaie, S., M. Mirzaei and J. Paterson, 2010. Bread improvers: Comparison of a range of lipases with a traditional emulsifier. Food Chem., 122: 495-499.

Mongkolthanaruk, W. and S. Dharmsthiti, 2002. Biodegradation of lipid-rich waster water by a mixed bacterial consortium. Int. Biodeterioration Biodegrad., 50: 101-105.

Mormeneo, M., I. Andres, C. Bofill, P. Diaz and J. Zueco, 2008. Efficient secretion of *Bacillus subtilis* lipase A in *Saccharomyces cerevisiae* by translational fusion to the Pir4 cell wall protein. Appl. Microbiol. Biotechnol., 80: 437-445.

Murty, V.R., J. Bhat and P.K.A. Muniswaran, 2002. Hydrolysis of oils by using immobilized lipase enzyme: A review. Biotechnol. Bioprocess Eng., 7: 57-66.

Nair, S. and P. Kumar, 2007. Molecular characterization of a lipase-producing *Bacillus pumilus* strain (NMSN-1d) utilizing colloidal water-dispersible polyurethane. World J. Microbiol. Biotechnol., 23: 1441-1449.

Nandini, K.E. and N.K. Rastogi, 2009. Reverse micellar extraction for downstream processing of lipase: Effect of various parameters on extraction. Process Biochem., 44: 1172-1178.

Nawani, N. and J. Kaur, 2000. Purification, characterization and thermostability of a lipase from thermophilic *Bacillus sp.* Mol. Cell. Biochem., 206: 91-96.

Nawani, N. and J. Kaur, 2007. Study on lipolytic isoenzymes from a thermophilic *Bacillus* sp.: Production, purification and biochemical characterization. Enzyme Microb. Technol., 40: 881-887.

Nelson, D.J. and R. Rawson, 2010. Method of growing bacteria for use in wastewater treatment. United States Patent 7658851. www.freepatentsonline.com

Ooi, C.W., B.T. Tey, S.L. Hii, S.M.M. Kamal, J.C.W. Lan, A. Ariffe and T.C. Ling, 2009. Purification of lipase derived from *Burkholderia pseudomallei* with alcohol/salt-based aqueous two-phase systems. Process Biochem., 44: 1083-1087.

Padilha, G.S., J.C. Curvelo-Santano, R.M. Alegre and E.B. Tambourgi, 2009. Expanded bed adsorption of an alkaline lipase from *Pseudomona cepacia*. J. Chromatogr. B., 877: 521-526.

Pandey, A., S. Benjamin, C.R. Soccol, P. Nigam, N. Krieger and V.T. Soccol, 1999. The realm of microbial lipases in biotechnology. Biotechnol. Appl. Biochem., 29: 119-131.

Pauwels, K. and P.V. Gelder, 2008. Affinity based isolation of a bacterial lipase through steric chaperone interactions. Protein Expr. Purif., 59: 342-348.

Pogaku, P., A. Suresh, P. Srinivas and S.R. Reddy, 2010. Optimization of lipase production by *Staphylococcus* sp. Lp12. Afr. J. Biotechnol., 9: 882-886.

Priya, K. and A. Chadha, 2003. Synthesis of hydrocinnamic esters by *Pseudomonas cepacia* lipase. Enzyme Microb. Technol., 32: 885-890.

Quax, W.J., 2006. Bacterial Enzymes. In: The Prokaryotes: Symbiotic Associations, Biotechnology, Applied Microbiology, Dworkin, M. and S. Falkow (Eds.), Springer, New York, NY, pp: 777-796,.

Quyen, D.T., C. Schmidt-Dannert and R.D. Schmid, 2003. High-level expression of a lipase from *Bacillus thermocatenulatus* BTL2 in *Pichia pastoris* and some properties of the recombinant lipase. Protein Expr. Purif., 28: 102-110.

Rahman, R.N.Z.A., L.P. Geok and M. Basri, 2005. Physical factors affecting the production of organic solvent-tolerant protease by *Pseudomonas aeruginosa*. Bioresource Technol., 96: 429-436.

Rahman, R.N.Z.R.A, A.B. Salleh and M. Basri, 2006. Lipases: Introduction. In: New Lipases and Proteases, Salleh, A.B., R.N.Z.R.A. Rahman and M. Basri (Eds.), Nova Science Publishers, New York. ISBN-1-60021-068-6, pp: 1-22.

Raita, M., V. Champreda, N. Laosiripojana, 2010. Biocatalytic ethanolysis of palm oil for biodiesel production using microcrystalline lipase in tert-butanol system. Process Biochem., 45: 829-834.

Rajmohan, S., C.E. Dodd and W.M. Waites, 2002. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. J. Applied Microbiol., 93: 205-213.

Ramani, K., E. Chockalingam and G. Sekaran, 2010. Production of a novel extracellular acidic lipase from *Pseudomonas gessardii* using slaughterhouse waste as a substrate. J. Ind. Microbiol. Biotechnol., 37: 531-535.

Ramchuran, S.O., V.A. Vargas, R. Hatti-Kaul and E.N. Karlsson, 2006. Production of a lipolytic enzyme originating from *Bacillus halodurans* LBB2 in the methylotrophic yeast *Pichia pastoris*. Appl. Microbiol. Biotechnol., 71: 463-472.

Rathi, P., R.K. Saxena and R. Gupta, 2001. A novel alkaline lipase from *Burkholderia cepacia* for detergent Formulation. Process Biochem., 37: 187-192.

Riaz, M., A.A. Shah, A. Hameed and F. Hasan, 2010. Characterization of lipase produced by *Bacillus* sp. FH5 in immobilized and free state. Ann. Microbiol., 60: 169-175.

Rosenau, F., J. Tommassen and K.E. Jaeger, 2004. Lipase specific foldases in bacteria. Chem. Biochem., 5: 152-161.

Rosenstein, R. and F. Gotz, 2000. Staphylococcal lipases: Biochemical and molecular characterization. Biochimie, 82: 1005-1014.

Ruchi, G., G.N. Gupta, M. Vamsikrishnan and S.K. Khare, 2008a. Protein coated microcrystals of *Pseudomonas aeruginosa* PseA lipase. Appl. Biochem. Biotechnol., 151: 160-166.

Ruchi, G., A. Gupta and S.K. Khare, 2008b. Lipase from solvent tolerant *Pseudomonas aeruginosa* strain: Production optimization by response surface methodology and application. Bioresour. Technol., 99: 4796-4802.

Sakai, S., Y. Liu, T. Yamaguchi, R. Watanabe, M. Kawabe and K. Kawakami, 2010. Immobilization of *Pseudomonas cepacia* lipase onto electrospun polyacrylonitrile fibers through physical adsorption and application to transesterification in nonaqueous solvent. Biotechnol. Lett., 10.1007/s10529-010-0279-8

Salis, A., M.S. Bhattacharyya, M. Monduzzi and V. Solinas, 2009. Role of the support surface on the loading and the activity of *Pseudomonas fluorescens* lipase used for biodiesel synthesis. J. Mol. Catal. B. Enz., 57: 262-269.

Sanchez, M., N. Prim, F. Rendez-Gil, F.I.J. Pastor and P. Diaz, 2002. Engineering of baker's yeasts, *Escherichia coli* and *Bacillus* hosts for the production of *B. subtilis* lipase A. Biotechnol. Bioeng., 78: 339-345.

Sangeetha, R., A. Geetha and I. Arulpandi, 2010a. Concomitant production of protease and lipase by *Bacillus licheniformis* VSG1: Production, purification and characterization. Brazilian J. Microbiol., 41: 179-185.

Sangeetha, R., A. Geetha and I. Arulpandi, 2010b. Concomitant production, partial purification and characterization of a serine protease and a proteolysis-resistant metallolipase from *Bacillus pumilus* SG2. Z. Naturforsch, 65: 61-65.

Sani, D.G., 2006. Step up: Bacterial lipase to substitute pancreatic lipase for enzymetherapy. www.microbepundit.blogspot.com

Saxena, R.K., A. Sheoran, B. Giri and W.S. Davidson, 2003. Purification strategies for microbial lipases. J. Microbiol. Methods., 52: 1-18.

Schmidt-Dannert, C., 1999. Recombinant microbial lipases for biotechnological applications. Bioorg. Med. Chem., 7: 2123-2130.

Setzu, S., S. Salis, V. Demontis, A. Salis, M. Monduzzi and G. Mula, 2007. Porous silicon-based potentiometric biosensor for triglycerides. Phys. Status solidi (a)., 204: 1434-1438.

Sharma, R., S.K. Soni, R.M. Vohra, L.K. Gupta and J.K. Gupta 2002. Purification and characterisation of a thermostable alkaline lipase from a new thermophilic *Bacillus* sp. RSJ-1. Process Biochem., 37: 1075-1084.

Shukla, P., K. Gupta and N. Kango, 2007. Production of lipase by hyper-lipolytic *Rhizopus oryzae* KG-10 on low-value oil emulsions. Res. J. Microbiol., 2: 671-677.

Singh, R., N. Gupta, V.K. Goswami and R. Gupta, 2006. A simple activity staining protocol for lipases and esterases. Appl. Microbiol. Biotechnol., 70: 679-682.

Singh, M., R.S. Singh and U.C. Banerjee, 2010. Enantioselective transesterification of racemic phenyl ethanol and its derivatives in organic solvent and ionic liquid using *Pseudomonas aeruginosa* lipase. Process Biochem., 45: 25-29.

Suzuki, M, 2001. Alkaline lipase and detergent composition active at low temperature. United States patent 6306813. http://www.freepatentsonline.com

Talon, R., M.C. Montel and J.L. Berdague, 1996. Production of flavour estes by lipases of *Staphylococcus warneri* and *Staphylococcus xylosus*. Enzyme Microb, Technol., 19: 620-622.

Tancous, J.J., W.E. Kallenberger, B.W. Buehrig and L.S. Herman, 1994. A study of various types of soaks on the rehydration of brine cured hides used for vegetable tanned leather. J. Am. Leather Chem. Assoc., 89: 241-241.

Taneja, S.C., V.K. Sethi, S.S. Andotra, S. Koul and G.N. Qazi, 2005. Rose oxides: A facile chemo and chemo-enzymatic approach. Synth. Commun., 35: 2297-2303.

Thanikaivelan, P., J.R. Rao, B.U. Nair and T. Ramasami, 2004. Progress and recent trends in biotechnological methods for leather processing. Trends Biotech., 22: 181-188.

Tripathi, M.K, U. Roy, U.K. Jinwal, S.K. Jain and P.K. Roy, 2004. Cloning, sequencing and structural features of a novel *Streptococcus* lipase. Enzyme Microb. Technol., 34: 437-442.

Vujaklija, D., M. Abramic, I. Lescic, T. Marsic and J. Pigac, 2003. *Streptomyces rimosus* GDS(L) lipase: Production, Heterologous overexpression and structure-stability relationship. Food Technol. Biotechnol., 41: 89-94.

Wang, Q., L. Su, X. Fan, J. Yuan, L. Cui and P. Wang, 2002. Effects of Lipase on poly(lactic acid) fibers. Fibers Polym., 10: 333-337.

Wang, X., X. Yu and Y. Xu, 2009. Homologous expression, purification and characterization of a novel high-alkaline and thermal stable lipase from *Burkholderia cepacia* ATCC 25416. Enzyme Microb. Technol., 45: 94-102.

Wang, Y. and L. Zhang, 2009. Ectoine improves yield of biodiesel catalyzed by immobilized lipase. J. Mol. Catal. B. Enzym., 62: 91-96.

Westers, H., P.G. Braun, L. Westers, H. Antelmann and M. Hecker *et al.*, 2005. Genes involved in SkfA killing factor production protect a *Bacillus subtilis* lipase against proteolysis. Appl. Environ. Microbiol., 71: 1899-1908.

Wu, H.S. and M.J. Tsai, 2004. Kinetics of tributyrin hydrolysis by lipase. Enzyme Microb. Technol., 35: 488-493.

Xu, J.H., R. Zhou and U.T. Bornscheuer, 2005. Comparison of differently modified *Pseudomonas cepacia* lipases in enantioselective preparation of a chiral alcohol for agrochemical use. Biocatal. Biotransform., 23: 415-422.

Xu, Y., H. Liu, W. Du, Y. Sun, X. Ou and D. Liu, 2009. Integrated production of biodiesel and 1,3-propanediol with lipase catalysed transesterification and fermentation. Biotechnol. Lett., 39: 1335-1341.

Yamauchi, A., T. Nagao, Y. Watanabe, M. Sumida, T. Kobayashi and Y. Shimada, 2005. Purification of arachidonic acid from *Mortierella* single cell oil by selective esterification with *Burkholderia cepacia* lipase. J. Am. Oil Chem. Soc., 82: 833-837.

Yan, J., L. Li, Q. Tang, M. Jiang and S. Jiang, 2010. Preparation of a crosslinked bioimprinted lipase for enrichment of polyunsaturated fatty acids from fish processing waste. Appl. Biochem. Biotechnol., 162: 757-765.

Yang, J., B. Zhang and Y. Yan, 2009. Cloning and expression of *Pseudomonas fluorescens* 26-2 lipase gene in *Pichia pastoris* and characterizing for transesterification. Appl. Biochem. Biotechnol., 159: 355-365.

Yilmaz, E., 2002. Bio imprinting of microbial lipase at air water interface. World J. Microbiol. Biotechnol., 18: 141-145.

Yilmaz, E., 2003. Orlistat induced molecular bio imprinting of microbial lipase. World J. Microbiol. Biotechnol., 19: 161-165.

Zarringhalami, S., M.A. Sahari, M. Barzegar and Z. Hamidi-Esfehani, 2010. Enzymatically modified tea seed oil as cocoa butter replacer in dark chocolate. Int. J. Food Sci. Technol., 45: 540-545.

Zhang, Y., K. Menga, Y. Wang, H. Luo and P. Yang *et al.*, 2008. A novel proteolysis-resistant lipase from keratinolytic *Streptomyces fradiae* var. k11. Enzyme Microb. Technol., 42: 346-352.

Zhang, A., R. Gao, N. Diao, G. Xie, G. Gao and S. Cao, 2009a. Cloning, expression and characterization of an organic solvent tolerant lipase from *Pseudomonas fluorescens* JCM5963. J. Mol. Catal. B. Enzym., 56: 78-84.

Zhang, H., F. Zhang and Z. Li, 2009b. Gene analysis, optimized production and property of marine lipase from *Bacillus pumilus* B106 associated with South China Sea sponge *Halichondria rugosa*. World J. Microbiol. Biotechnol., 25: 1267-1274.

Zhao, L.L, J.H. Xu, J. Zhao, J. Pan and Z.L. Wang, 2008. Biochemical properties and potential applications of an organic solvent tolerant lipase isolated from *Serratia marcescens* ECU1010. Process Biochem., 43: 226-233.