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Original article

Applications of Extracellular Microbial Lipase- A Review

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Abstract
Lipase, a classic lipolytic enzyme is indispensable for the biological turnover of lipids. Lipase (triacyl glycerol acylhydrolases, EC 3.1.1.3) catalyzes the hydrolysis of lipids to fatty acid and glycerol and various biotransformation reactions, such as interesterification, transesterification, alcoholysis, acidolysis and aminolysis. Commercial significance of lipases from microbial resources is well established. Biotechnological applications of lipase are suitably exploited. Novel lipase applications, like biopolymer synthesis, biodiesel production, treatment of fat-containing waste effluents, synthesis of pharmaceuticals and nutraceutical agents including food processing sectors have been established successfully. Nowadays immobilization process can provide the ability to confine lipase in a well-defined predetermined space which creates an opportunity for applications. In view of the increasing demand of lipases and their versatile applications, the present paper emphasized on this enzyme towards increasing impact on food and bioprocessing.

Keywords: Microbial lipase, biotransformation, synthesis, interesterification, immobilization

1. Introduction
Lipases have attracted much interest in enzyme technology in recent years. This is partly because modification of the natural substrates of the lipases, triacylglycerol is of great technical interest. However another important aspect is that lipases often have quiet broad substrate specificity and therefore can be used for the conversion of many unnatural substrates [1]. Lipases are glycerol ester hydrolases (E.C. 3.1.1.3), which hydrolyze ester linkages of glycerides at water-oil interface. During hydrolysis lipases pick acyl group from glycerides forming lipase-acyl complex, which then transfers its acyl group to OH group of water. However, in non-aqueous conditions, these naturally hydrolytic enzymes can transfer acyl groups of carboxylic acids to nucleophiles other than water [2]. Thus lipases can acylate alcohols, sugars, thiols and amines synthesizing a variety of stereo-specific esters, sugar esters, thioesters and amides[3,4]. Lipases are indispensable for the bioconversion of lipids (triacylglycerols) from one organism to another and within the organisms, and they possess the unique feature of acting at an interface between the aqueous and nonaqueous(i.e. organic) phase; this feature distinguishes them from esterase [5]. Lipases are produced by animals, plants, and microorganisms. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity [6,7]. Many microorganisms are known as potential producers of extracellular lipases, including bacteria, yeast, and fungi [8]. Fungal species are preferably cultivated in solid-state fermentation (SSF), while bacteria and yeast are cultivated in submerged fermentation [9]. Today the most widely used lipase assay protocol is the titrimetry assay using olive oil as a substrate followed by author [10] because of its simplicity, accuracy and reproducibility. The ability of lipases to perform very specific chemical transformation (biotransformation) has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [11-14]. The number of available lipases has increased since the 1980s. This is mainly a result of the huge achievements made in the cloning and expression of enzymes from microorganisms, as well as of an increasing demand for these biocatalysts with novel and specific properties such as specificity, stability, pH, and temperature [15, 16]. Immobilization techniques are capable of entrapping the biocatalyst particle on suitable matrix for repeated use and effective substrate conversion [17].

2. Overview of lipase applications
Development of lipase-based technologies for the synthesis of novel compounds is rapidly expanding the uses of these
enzymes [18,19]. Lipases can play an important role in the processing of linolenic acid, a polyunsaturated fatty acid (PUFA); astaxanthine, a food colorant; methylketones, flavor molecules characteristic of blue cheese; 4-hydroxydecanoic acid used as a precursor of g-decalactone, a fruit flavor; dicarboxylic acids for use as prepolymers; interesterification of cheaper glycerides to more valuable forms e.g., cocoa butter replacements for use in chocolate manufacture [20]. The ability of lipases to perform very specific chemical transformation (biotransformation) has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [11,13], food and agro-chemical industries e.g., processing foods, treatment of fatty effluents, synthesis of biosurfactants, removal of resin (pitch) in processing paper from wood cellulose pulps, and as biocatalyst in biotransformation reactions in the semi synthesis of drugs [21]. The increasing awareness of the importance of chirality in the context of biological activity has stimulated a growing demand for efficient methods for industrial synthesis of pure enantiomers including chiral anti-inflammatory drugs such as naproxen and ibuprofen [22-26]; antihypertensive agents such as angiotensin-converting enzyme (ACE) inhibitors (e.g., captopril, enalapril, ceranopril, zofenapril, and lisinopril); and the calcium channel blocking drugs such as diltiazem. Lipases are used in synthesis of these drugs [27]. Lipase for use in detergents needs to be thermostable and remains active in the alkaline environment of a typical machine wash. An estimated 1000 tons of lipases are added to approximately 26 billion tons of detergents produced each year [28]. The main industrial application of lipase is still restricted to their use in laundry detergents to remove fats and oil stains.

Table -1: Industrial applications of microbial lipases [41]

<table>
<thead>
<tr>
<th>Industry</th>
<th>Action</th>
<th>Product or application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergents</td>
<td>Hydrolysis of fats</td>
<td>Removal of oil stains from fabrics</td>
</tr>
<tr>
<td>Dairy foods</td>
<td>Hydrolysis of milk fat, cheese ripening, modification of butter fat</td>
<td>Development of flavoring agents in milk, cheese, and butter</td>
</tr>
<tr>
<td>Bakery foods</td>
<td>Flavor improvement</td>
<td>Shelf-life prolongation</td>
</tr>
<tr>
<td>Beverages</td>
<td>Improved aroma</td>
<td>Beverages</td>
</tr>
<tr>
<td>Food dressings</td>
<td>Quality improvement</td>
<td>Mayonnaise, dressings, and whippings</td>
</tr>
<tr>
<td>Health foods</td>
<td>Transesterification</td>
<td>Health foods</td>
</tr>
<tr>
<td>Meat and fish</td>
<td>Flavor development</td>
<td>Meat and fish products; fat removal</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>Transesterification; hydrolysis</td>
<td>Cocoa butter, margarine, fatty acids, glycerol, mono-, and diglycerides</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Enantioselectivity, synthesis</td>
<td>Chiral building blocks, chemicals</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Transesterification, hydrolysis</td>
<td>Specialty lipids, digestive aids</td>
</tr>
<tr>
<td>Cosmetics</td>
<td>Synthesis</td>
<td>Emulsifiers, moisturizers</td>
</tr>
<tr>
<td>Leather</td>
<td>Hydrolysis</td>
<td>Leather products</td>
</tr>
<tr>
<td>Paper</td>
<td>Hydrolysis</td>
<td>Paper with improved quality</td>
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<tr>
<td>Cleaning</td>
<td>Hydrolysis</td>
<td>Removal of fats</td>
</tr>
</tbody>
</table>

2.1 Lipases in food processing industry

Fats and oils are important constituents of foods. The nutritional and sensory value and the physical properties of a triglyceride are greatly influenced by factors such as the position of the fatty acid in the glycerol backbone, the chain length of the fatty acid, and its degree of unsaturations. Lipases are able to modify the properties of lipids by altering the location of fatty acid chains in the glyceride and replacing one or more of the fatty acids with new ones. This way, a relatively inexpensive and less desirable lipid can be modified to a higher value fat. [42,43]. Cocoa butter, a high-value fat, contains palmitic and stearic acids and has a melting point of approximately 37°C. Melting of cocoa butter in the mouth produces a desirable cooling sensation in products such as chocolate. Lipase-based technology involving mixed hydrolysis and synthesis reactions is used commercially to upgrade some of the less desirable fats to cocoa butter substitutes [43,44]. One version of this process uses immobilized Rhizomucor
miehei lipase for the trans esterification reaction that replaces the palmitic acid in palm oil with stearic acid. Similarly [44] described a lipase-catalyzed interesterification of butter fat that resulted in a considerable decrease in the long-chain saturated fatty acids and a corresponding increase in C18:0 and C18:1 acids at position 2 of the selected triacylglycerol. Because of their metabolic effects, PUFAs are increasingly used as pharmaceuticals, nutraceuticals, and food additives [45,46]. Microbial lipases are used to obtain PUFAs from animal and plant lipids such as menhaden oil, tuna oil, and borage oil. Free PUFAs and their mono- and diglycerides are subsequently used to produce a variety of pharmaceuticals including anticholesterolemic, anti-inflammatory and thrombotic [45,46]. In addition, lipases have been used for development of flavors in cheese ripening, bakery products, and beverages [47]. Also, lipases are used to aid removal of fat from meat and fish products [47].

2.2 Lipases in pulp and paper industry
Pitch, the hydrophobic components of wood (mainly triglycerides and waxes), causes severe problems in pulp and paper manufacture [29]. Lipases are used to remove the pitch from the pulp produced for paper making. Nippon Paper Industries, Japan, have developed a pitch control method that uses the Candida rugosa fungal lipase to hydrolyze up to 90% of the wood triglycerides [48].

2.3 Lipases in organic synthesis
Use of lipases in organic chemical synthesis is becoming increasingly important. Lipases are used to catalyze a wide variety of chemo-, regio-, and stereo selective transformations [27, 47, 49]. Majority 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 discussed by [27]. The enzymes catalyze the hydrolysis of water-immiscible triglycerides at water–liquid interface. Under given conditions, the amount of water in the reaction mixture will determine the direction of lipase-catalyzed reaction. When there is little or no water, only esterification and transesterification are favored [51]. Hydrolysis is the favored reaction when there is excess water [50]. Lipase-catalyzed reactions in supercritical solvents have been described [51, 52, 53].

2.4 Lipases in bioconversions in aqueous media
Hydrolysis of esters is commonly carried out using lipase in two-phase aqueous media [54, 55], [56] reported on the hydrolysis of p-nitro phenyl palmitate (pNPP) in n-heptane by a lipase preparation of P. cepacia. [29] used lipase entrapped in a hydrophobic sol–gel matrix for a variety of transformations. Mutagenesis has been used to greatly enhance the enantioselectivity of lipases [57, 58]. For example, in one case, the enantioselectivity of lipase catalyzed hydrolysis of a chiral ester (P. aeruginosa lipase) was increased from e.e. 2% to e.e. 81% in just four mutagenesis cycles. The lipase-acyl transferase from C. parapsilosis has been shown to catalyze fatty hydroxamic acid biosynthesis in a biphasic liquid/aqueous medium [59]. The substrates of the reaction were acyl donors (fatty acid or fatty acid methyl ester) and a hydroxylamine. The transfer of acyl group from a donor ester to hydroxylamine (aminolysis) was catalyzed preferentially compared to the reaction of free fatty acids. This feature made the C. parapsilosis enzyme the catalyst of choice for the direct bioconversion of oils in aqueous medium [55, 60] reported a novel lipase produced by Burkholderia sp., which could preferentially hydrolyze a bulky ester, t-butyloctanoate (TBO). This lipase was confirmed to be 100-fold superior to commercial lipases in terms of its TBO-hydrolyzing activity.

2.5 Lipases in bioconversions in organic media
Enzymes in organic media without a free aqueous phase are known to display useful unusual properties, and this has firmly established nonaqueous enzyme systems for synthesis and biotransformation [50]. Lipases have been widely investigated for various nonaqueous biotransformations [22, 60–64].

2.6 Lipases in resolution of racemic acids and alcohols
Stereo selectivity of lipases has been used to resolve various racemic organic acid mixtures in immiscible biphasic systems [62]. Racemic alcohols can also be resolved into enantiomerically pure forms by lipase-catalyzed transesterification. [65] reported that esterification reaction in nonaqueous media using lipase-B from C. antarctica was stereo selective towards the R-isomer of ketoprofen in an achiral solvent such as isobutyl methyl ketone and (S+)-carvone. In one study, a purified lipase preparation from C. rugosa was compared to its crude counterpart in anhydrous and slightly hydrated hydrophilic organic solvents. The purified lipase preparation was less active than the crude enzyme in dry n-heptane, whereas the presence of a small concentration of water dramatically activated the purified enzyme but not the crude enzyme in the esterification of racemic 2-(4-chlorophenoxy) propanoic acid with butanol [65]. Profens (2-aryl propionic acids), an important group of nonsteroidal antiinflammatoridrugs, are pharmacologically active mainly in the (S)-enantiomer form [66]. For instance, (S)-ibuprofen [(S)-2-(4-isobutylphenyl)propionic acid] is 160 times more potent than its antipode in inhibiting prostaglandin synthesis. Consequently, considerable effort is being made to obtain optically pure profens through asymmetric chemical synthesis, catalytic kinetic resolution [67, 68], resolution of racemate via crystallization, and chiral chromatographic separations. Microorganisms and enzymes have proved particularly useful in resolving racemic mixtures. Thus, pure (S)-ibuprofen is obtained by using lipase-catalyzed kinetic resolution via hydrolysis [69] or esterification [22, 70]. Similarly, 2-phenoxy-1-propanol was resolved into its enantiomers using Pseudomonas sp. lipase by enantioselective transesterification [71, 72] reported solvent-free thioesterification fatty acids with long-chain thiols catalyzed by lipases from C. antarctica and R. miehei. Also, solvent-free trans-thioesterification of fatty acid methyl esters with alkane thiols was reported [72].

2.7 Lipases in regioselective acylations
Lipases acylate certain steroids, sugars, and sugar derivatives with a high regioselectivity.
Monoacylated sugars have been produced in anhydrous pyridine from triethyl carboxylates and various monosaccharides [60]. In contrast [73,74] used a lipase from \textit{A. niger} to catalyze the regioselective decylation of preacylated methylB-D-glucopyranoside. Similarly [75] reported regioselective decacylation of preacylated monosaccharide derivatives in 1, 1, 1-trichloroethane using a lipase modified with polyethylene glycol.

2.8 Lipases in ester synthesis

Lipases have been successfully used as catalysts for the synthesis of esters. The esters produced from short-chain fatty acids have applications as flavoring agents in food industry [41]. Methyl and ethyl esters of long-chain acids have been used to enrich diesel fuels [41]. [75] studied the esterification of lactic acid and alcohols using a lipase of \textit{C. antarctica} in hexane. Esterification of five positional isomers of acetylenic fatty acids (different chain lengths) with n-butanol was studied by [76], using eight different lipases. [26] noted that an optimum preequilibration water activity value was necessary for obtaining a high rate of esterification of (R, S)-ibuprofen. [77] reported on the esterification of sulcatol and fatty acids in toluene, catalyzed by \textit{C. rugosa} lipase [78] reported using lipase immobilized on silica and microemulsion-basedorganogels, for ester synthesis.

2.9 Lipases in oleo chemical industry

Use of lipases in oleo chemicals processing saves energy and minimizes thermal degradation during alcoholysis, acidolysis, hydrolysis, and glycerolysis [41,57]. Although lipases are designed by nature for the hydrolytic cleavage of the ester bonds of triacylglycerols, lipases can catalyze the reverse reaction (ester synthesis) in a low water environment. Hydrolysis and esterification can occur simultaneously in a process known as interesterification. Depending on the substrates, lipases can catalyze acidolysis (where an acyl moiety is displaced between an acyl glycerol and a carboxylic acid), alcoholysis (where an acyl moiety is displaced between an acyl glycerol and an alcohol), and transesterification (where two acyl moieties are exchanged between two acyglycerols)[79].

2.10 Lipases in the detergent industry

Because of their ability to hydrolyze fats, lipases find a major use as additives in industrial laundry and household detergents. Detergent lipases are especially selected to meet the some specific requirements e.g. a low substrate specificity, i.e., an ability to hydrolyze fats of various compositions, ability to withstand relatively harsh washing conditions (pH 10–11,30–60°C temperature), ability to withstand damaging surfactants and enzymes (e.g., linear alkyl benzene sulfonates (LAS) and proteases) which are important ingredients of many detergent formulations. Lipases with the desired properties are obtained through a combination of continuous screening [80] and protein engineering [47].

2.11 Lipases as biosensors

A promising new field is the use of microbial lipase as biosensors. A biosensor is an analytical device that uses biomaterials as elements of the sensing system and converts biological response into an electrical signal. A biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element that is in direct spatial contact with a transduction element [81]. The polymer enzyme system: poly (trimethylene) succinate, was investigated or use in the sensor, which is degraded by a lipase. Potential fields of application of such a sensor system are the detection of enzyme concentrations and the construction of disposable enzyme based immunosensors, which employ the polymer degrading enzyme as an enzyme label [82]. A diolabelled polynucleotide probes have been employed extensively for the detection of complementary nucleic acids by specific hybridization. Within the last few years, various methods have been developed using enzyme-labeled probes to void unstable and hazardous isotopes. By screening various hydrolytic enzymes to fit the special demands, fungal lipases turned out to be the most practical [83]. Lipases may be immobilized onto pH/oxygen electrodes in combination with glucose oxidase, and these function as lipid biosensors [84] and may be used in triglycerides [85] and blood cholesterol determinations [86]. Following is the lipase catalyzed reactions [87] involving the release or absorption of H+ ions that may be utilized by the proposed potentiometric lipase-biosensor (figure-1). A porous silicon-based potentiometric biosensor constituted by a \textit{Candida rugosa} lipase immobilized on a mesoporous Si matrix can be used for the detection of triglycerides [88]. A novel glass electrode based potentiometric lipase using olive oil as substrate can be effectively demonstrated high activity and stability.

\textbf{Figure 1: Lipolysis of biodiesel glyceride component}

![Image](305x388 to 545x425)

\textbf{Glycerides in biodiesel + H}_2\textbf{O} \rightarrow \textbf{Glycerol+ Fatty acids + H+}

2.11.1 Lipases as Biosensors for Food Industry

Immobilized lipases are fast, efficient, accurate and cost effective as sensors for the quantitative determination of triacylglycerols. This application is important in the food industry, especially in fats and oils, beverages, soft drinks, pharmaceutical industries and also in clinical diagnosis. The basic concept of using lipase as biosensors is to generate glycerol from the triacylglycerols in the analytical sample and to quantify the released glycerol by a chemical or enzymatic method. [90] developed a method for the determination of organ phosphorous pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis. This method is also used to determine the dichlorovinyl residues in the root, stem and blade of Chinese cabbage. Lipases may be immobilized onto pH/oxygen electrodes in combination with glucose oxidase, and these function as lipid biosensors and may be used in triglycerides and blood cholesterol determinations [89].

2.12 Lipases for Pharmaceutical Application

Microbial lipases are used to enrich PUFAs from animal and plant lipids, and their mono and diacylglycerides are used to produce a variety of pharmaceuticals [63]. PUFAs are increasingly used as food additives, pharmaceuticals and nutraceuticals because of their metabolic benefits. Many 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. Free PUFAs and their mono and diacylglycerides are subsequently used to produce a variety of pharmaceuticals. Liposomes are used in the medical field to optimize the action of drugs by transporting them to target areas, thus circumventing drug waste inactivation and anatomical barriers. Considerable effort is being made to obtain optically, pure compounds, which are pharmacologically more active than its antipode. Profens, a class of nonsteroidal anti-inflammatory drugs, are active in the (S)-enantiomer form. [69] and [91] synthesized pure (S)-ibuprofen using lipase catalyzed kinetic resolution via hydrolysis and esterification, respectively. In addition to racemization in situ, lipases are also capable of catalyzing synthetic reactions, which has led to the production of life saving drugs. Efficient kinetic resolution processes are available for the preparation of optically active homochiral intermediates for the synthesis of nikkomyacin-B, nonsteroid anti-inflammatory drugs (naproxen, ibuprofen, suprofen and ketoprof), the potential anti viral agent lamivudine, and for the enantiospecific synthesis of alkaloids, antibiotics, vitamins, and antiarteriosclerotic, anti tumor and antiallergic compounds. Nutraceuticals are food components that have health benefits beyond traditional nutritional value. Novel biotechnology tools, like immobilization, have also been applied for the isolation and incorporation of such food components in ordinary foods. Successful synthesis of nutraceuticals has been reported by employing immobilized lipases, such as those from C. antarctica and Lactobacillus ruteri [90]. Lipases are also used in the synthesis of the artificial sweetener sucralose by regioselective hydrolysis of octaacetylsucrose. Some limiting problems for such processes are insufficient enantioselectivity, limited enzyme activity, difficulties in recycling the lipase and inherent practical limitations of the kinetic resolution arising from the fact that 50% conversion is the maximum possible [90].

3. Application Summary

Microbial lipases are the potential biocatalysts due to its versatility. Most of the microbial specimens viz. bacteria, yeast, molds etc. are able to produce hydrolytic lipase. The tremendous potential of lipases in food and allied technology applications shows the need to develop novel cost-effective technologies for increased production, scaling up and purification of this versatile enzyme. Moreover diverse substrate utilization, enantioselectivity and stereo selective biotransformation proves its unique properties. Immobilization techniques are capable of entrapping the biocatalyst particle on suitable matrix for repeated use and effective substrate conversion [16]. Immobilized enzyme systems influences greatly on the processes involving catalytic transformations by lipases. Application of immobilized lipase as a biosensor is an effective tool for analyzing various parameters and demonstrated higher activity and stability. Mutagenesis, gene cloning, biochemical characterization has provided anew and valuable tool for improving or adapting enzyme properties to the desired requirements. Simultaneously, advances are being made in bioreactor and reaction technologies for effectively using the lipases. Therefore the rapid development of lipase technology will certainly helps to build up the novelty of application potential in future.

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