Fungal lipases: a review

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Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) occupy a prominent place among biocatalysts and carry out reactions in aqueous and non-aqueous media. They catalyze both the hydrolysis and synthesis of long chain acylglycerols. The chemo-, regio- and enantio- specific characteristics of lipase tend to be a focus research area for scientists and industrialists. Compared to plants and animals, microorganisms have been found to produce high amount of lipases. Fungal lipases stand out as the major sources of the enzyme because of their catalytic activity, low cost of production and relative ease in genetic manipulation. This review describes the various sources of lipases, their properties, purification methods, immobilization techniques, and potential industrial applications that make lipases to be biocatalysts of choice for the present and future. The aim of this review is to present recent information on fungal lipases.

Keywords: Microbial lipases; Enantio-selective; Trans-esterification; Production; Purification.

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Introduction

Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the million-dollar underexploited lipid technology bio-industry and have been used in situ lipid metabolism and ex situ multifaceted industrial applications [1]. Lipases are triacylglycerol acylhydrolases (E.C. 3.1.1.3) that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids. They are ubiquitous in nature and are produced by several plants, animals and microorganisms [2]. Microbial lipases have gained special industrial attention due to their ability to remain active under extremes of temperature, pH and organic solvents, and chemo-, regio and enantioselectivity. In addition to the hydrolysis of triglycerides, lipases can catalyze a variety of chemical reactions which include esterification, trans-esterification, acidolysis and aminolysis. Lipase is frequently used to catalyze the hydrolysis of wide non-natural substrates in order to obtain enantio- and regio selective substrates [3]. The numerous industrial applications of lipases have stimulated interest in isolation of new lipases from novel sources and strong efforts have been concentrated on the engineering of enzymes with specific properties or better performance for industrial applications [4]. The reasons for the enormous biotechnological potential of microbial lipases are: their stability in organic solvents, they do not require cofactors, possess broad substrate specificity and exhibit a high enantio-selectivity [5]. The high versatility of lipases allows their application in different industries like food, dairy [6], detergent [7], pharmaceutical [8, 9],

biodiesel production [10-12] leather, textile, cosmetic, paper and oleo-chemicals [13].

Among the microorganisms, fungi are recognized as one of the best lipase sources [14]. Fungal lipases today have gained significant attention in the industries due to their substrate specificity and stability under varied chemical and physical conditions. Fungal enzymes are extracellular in nature and they can be extracted easily, which significantly reduces the cost and makes this source preferable over bacteria. Soil contaminated with spillage from the products of oil and dairy harbors fungal species which have the potential to secrete lipases to degrade fats and oils [7]. The role of fungi in bioremediation process has been well documented [15]. There has been an increasing awareness of potentially harmful effects of the worldwide spillage of the oil and fatty substances in both saline and fresh waters. Domesticated waste is also considered as a pollutant as it has a high amount of fatty and oil substances and bioconversion by fungal activity results in the production of a vast number of useful substances. Filamentous fungi and yeasts usually behave more efficiently in solid-state fermentation and show greater productivities when compared to submerged fermentation [16]. Bearing this in mind, the present review is focused on fungal lipase production, properties and their wide range of industrial applications.

Historical Background

Lipase was first discovered in pancreatic juice in the year 1856 by Claude Bernard. Lipases were first demonstrated in plants seeds. Animal pancreatic extracts were traditionally used as the source of lipase for commercial applications. Lipase producers are widespread in the nature. 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. The first work on fungal lipases was reported by [17]. In 1994, Novo Nordisk introduced the first commercial recombinant lipase 'Lipolase' which originated from the fungus *Thermomyces lanuginosus* and was expressed in *Aspergillus oryzae*. Fungi capable of synthesizing lipases are found in several habitats, including soils contaminated with wastes of vegetable oils, dairy byproduct, seeds and deteriorated food [18, 19].

Classification of lipases

A new classification was more recently reported in the Lipase Engineering Database (LED) (http://www.led.uni-stuttgart.de), which today includes not only bacterial, but also yeast, fungal and mammalian lipases. This classification distributes the lipases into three classes on the basis of the oxyanion hole: GX, GGGX, and Y [20]. Based on this classification and of the amino-acid sequence similarities, yeasts and fungal lipases have been grouped into five different subclasses, two in the GX class, two in the GGGX class and one in the Y class (Figure 1) [21].

Sources

Lipases are ubiquitous enzymes and have been found mostly from the microbial [12, 14, 22-25], plant [26, 27] and animal kingdom [28]. Micro-organisms have the advantages including the ability to catalyze diverse reactions, produce high yields, broad substrate specificity, enhanced stability and reduced production costs [29, 30]. In addition, they have the advantage of relative ease of genetic manipulation. The interest in microbial lipase production has increased in the last decade, because of its large potential in manufacturing applications as food additives (flavor modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharma (digestion of oils and fats in foods), leather (removal of lipids from animal skins) and medicine (blood triglyceride assay) [9, 31-33]. Fungi have been considered as best lipase sources [34, 35] because of extracellular lipase production [16, 36]. Fungal lipases have benefits over bacterial ones due to the fact that present day technology favors the use of batch fermentation and low cost extraction methods. Major genera of filamentous fungi include *Rhizopus, Aspergillus,*

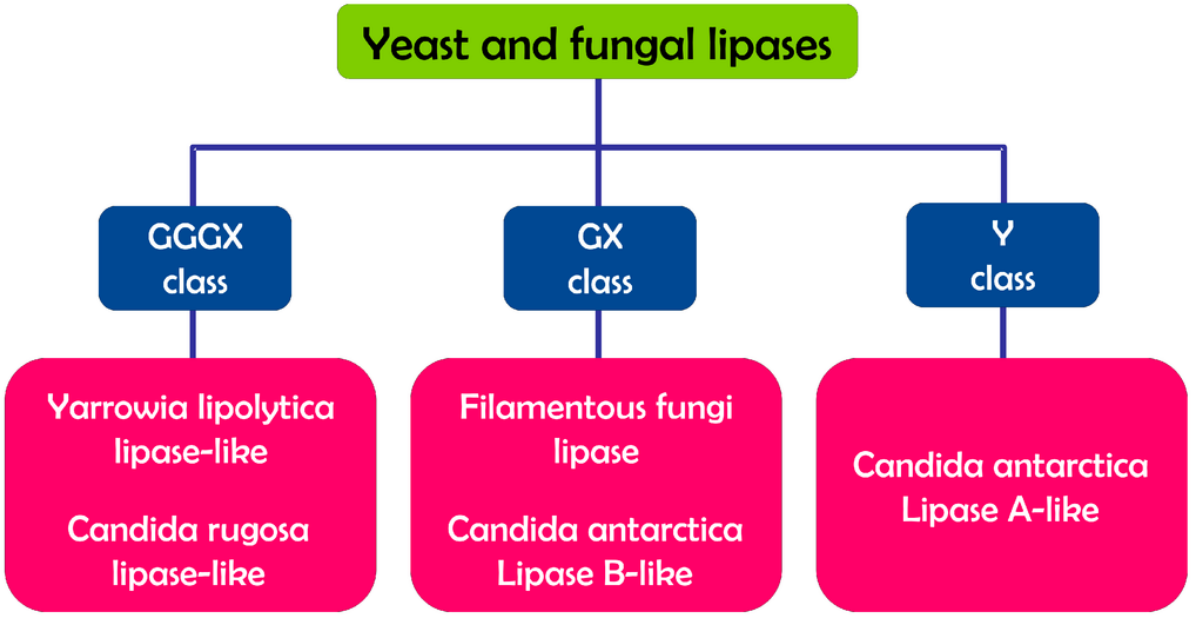

Figure 1. Classification of lipases based on lipase engineering database [21].

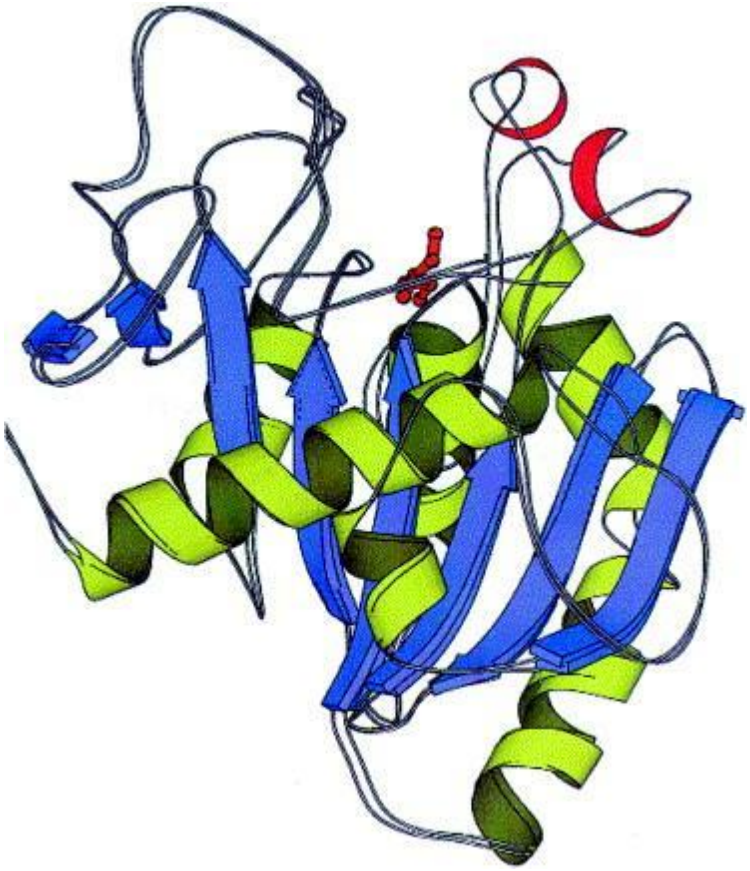

Figure 2. Crystal structure of the *Thermomyces lanuginosus* lipase. The β-sheet is shown in blue, surrounded by some helices in yellow, and the active serine site residue in red sticks, and the lid shown in red. Both the open and the closed conformation are shown superimposed [75].

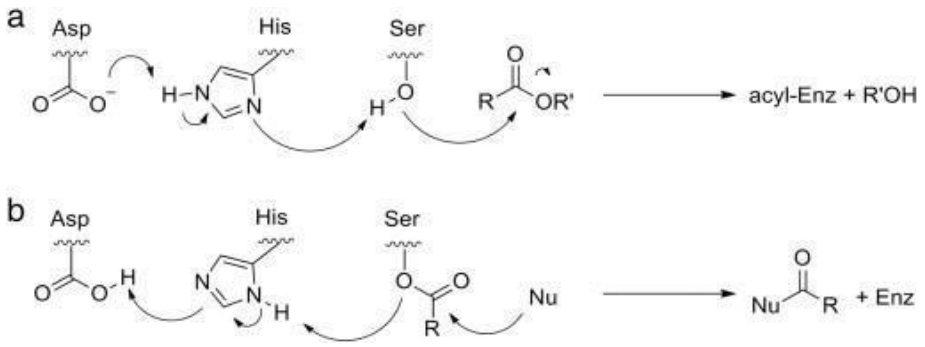

Figure 3. Steps of the lipase catalytic mechanism. An acyl-enzyme intermediate is formed by a serine nucleophilic attack on the carbonyl carbon, promoted by a histidine and an aspartate residue – these three amino acids form the catalytic triad – reaction **a**. This intermediate reacts with a nucleophile in a second nucleophilic attack, such as water, creating the product and returning the functional hydroxyl group to serine – reaction **b** [76].

Penicillium, Mucor, Ashbya, Geotrichum, Beauveria, Humicola, Rhizomucor, Fusarium, Acremonium, Alternaria, Eurotrium and *Ophiostoma* [37]. A list of various fungal lipase producers is presented in Table 1. Species of the mold *Aspergillus* are well known lipase producers. Lipases from *Aspergillus niger* are produced both intracellularly and extracellularly [54, 55]. Lipase from *Penicillium sp.* was optimized by using response surface methodology [56]. Fungal species which produce lipases are *Candida rugosa, Candida antarctica, T. lanuginosus, Rhizomucor miehei, Pseudomonas, Mucor* and *Geotrichum* [18, 57, 58]. Lipase-producing 59 fungal strains were isolated from Brazilian savanna soil by using enrichment culture techniques and among these *Colletotrichum gloesporioides* identified as most productive strain produced 27,700 U/L of lipase [59]. In another study, lipase from *Aspergillus sp*. having activity of 17 U/ml was reported [60].

Structure of lipase

The 3-D structures of lipases from *Rhizomucor miehei* [61, 62], *Geotrichum candidum* [63], *Candida rugosa* [64], *Rhizopus delemar* [65], *Pseudomonas glumae* [66], *Penicillium camembertii* [67], *Humicola lanuginosa* [68], and human pancreas [69] have been crystallographically resolved. These studies provide detailed insight into the structure-function relationships in lipases. The crystal structures indicated that all these lipases have a common α/β- hydrolase fold [70, 71]. It also showed a catalytic triad (Ser-His-Asp/Glu) similar to that found in serine proteases [72], and a lid covering the active site. During activation, the lid covering the active site gets displaced; this opens up the binding pocket, and the active site becomes accessible to the substrate. Lipases display a wide variety in primary sequences with a range of molecular masses from less than 20 kDa to about 60 kDa for larger fungal lipases, as in the case of *Geocthricum candidum* lipase. However, all lipases exhibit the same α/β-hydrolase fold structure [73], common also in many other hydrolases [74] and identical catalytic triad composed of Ser, His, Asp, and sometimes Glu in place of Asp (Figure 2) [75].

Catalytic mechanism of lipases

The mechanism of the lipase to catalyze ester hydrolysis is similar to carboxyl esterases and serine proteases, and involves a first nucleophilic attack of the serine on the carbonyl carbon of the ester bond, yielding a covalent acyl-enzyme intermediate and releasing an alcohol, i.e. a diacylglycerol would be released after forming a hydroxyl group in a triacylglycerol molecule [76] (Figure 3). This step is stabilized by the other two residues of the active site, histidine and aspartic acid. Then, a second nucleophilic attack occurs when the acyl-enzyme intermediate is hydrolyzed by water, finally forming a carboxylic

Table 1. Various fungal strains for lipase production through fermentation.

Microorganism	Time (h)	Lipase activity (U/ml)	Type of fermentation	Raw material	Reference
Penicillium aurantiogriseum	48	25	SmF	Soya bean oil	[38]
Rhizopus rhizopodiformis	24	43	SSF	Olive oil cake-Bagasse	[39]
Rhizopus pusillus	25	10.8	SSF	Olive oil cake-Bagasse	[39]
Penicillium restrictum	24	30	SSF	Babassu oil cake	[40]
Penicillium simplicissimum	36	30	SSF	Babassu oil cake	[41]
Rhizopus oligosporus TUV-31	48	76.6	SSF	Egg yolk	[42]
Rhizopus oligosporus ISUUV-16	48	81.2	SSF	Almond meal	[43]
Aspergillus carneu	96	12.7	SSF	Sunflower oil	[44]
Candida cylindracea	179.5	23.7	SmF	Oleic acid	[45]
Candida rugosa	50	3.8	SmF	Olive oil	[46]
Penicillium verrucosum	48	40	SSF	Soybean bran	[47]
Geotrichum sp.	24	20	SmF	Olive oil	[32]
Rhizopus homothallicus	12	826	SSF	Olive oil	[48]
Penicillium chrysogenum	168	46	SSF	Wheat bran	[49]
Fusarium solani FS1	120	0.45	SmF	Sesame oil	[50]
Penicillium simplicissimum	48	21	SSF	Soy cake	[51]
Aspergillus awamori	96	495	SmF	Rice bran oil	[52]
Candida cylindracea NRRLY-17506	175	20.4	SmF	Olive mill wastewater	[53]

acid. Many different compounds can act as acyl donors and likewise, in addition to water, many nucleophilic compounds can perform the same role and break the acyl-enzyme intermediate [77, 78]. Due to this broad substrate specificity, lipases can perform several reactions such as trans-esterification, esterification, interesterification, and acidolysis beyond their natural acylglycerol hydrolysis.

However, the catalytic options of lipases spread to several other synthetic or non-conventional substrates and types of reactions, from using amines as nucleophilic compounds to performing aldol additions [73, 79, 80].

Properties of lipases

The number of available lipases has increased since the 1980s and used as industrial biocatalysts because of their properties like biodegradability [81], high specificity [82], high catalytic efficiency [83], temperature [54], pH dependency, activity in organic solvents [84], and nontoxic nature. The most desired characteristics of the lipase are its ability to utilize all mono-, di-, and tri-glycerides as well as the free fatty acids in transesterification, low product inhibition, high activity/yield in non-aqueous media, low reaction time, resistance to altered temperature, pH, alcohol and reusability of immobilized enzyme. Additionally, lipases can carry out reactions under mild conditions of pH and temperature and this reduces energy required to direct reactions at unusual temperatures and pressures.

pH and temperature kinetics

Lipases are active over broad pH and temperature range [85]. They possess stability over a wide range from pH 4.0 to 11.0 and temperature optima in the range from 10 to 96°C. The extracellular lipase produced by *Aspergillus niger* and *Rhizopus arrhizus* are particularly active at low pH [86, 87]. Lipases

from *Aspergillus niger* [88] and *Rhizopus japonicas* [89] are stable at 50°C, and lipase of thermotolerant *Humicola lanuginosa* is stable at 60°C.

Substrates

Lipases catalyze various reactions since they have ability to act on wide range of substrates that may be artificial or natural [90]. Fungal lipases are extracellular and their production is influenced by nutritional and physicochemical factors such as temperature, pH, nitrogen, and carbon sources, and presence of lipids, inorganic salts, agitation rate, and dissolved oxygen concentration. Production of lipase can be significantly influenced by carbon sources such as sugars, sugar alcohol, polysaccharides, whey, amino acids and other complex sources [91-94]. Oleic acid (cis-9-Octadecenoic acid) has been reported as the most suitable inducer for the production of the main extracellular Lip2p lipase in *Yarrowia lipolytica* [95, 96]. The major factor for the expression of lipase activity has always been carbon source, since lipases are inducible enzymes. Palm oil mill effluent has been used for lipase production by *Candida cylindracea* with an activity of 20.26 U/ml under the optimized conditions [97]. Various mineral and organic nitrogen sources were tested for their capacity to support cell growth and lipase production [95]. Corn steep liquor, yeast extract, and peptone have been reported as best nitrogen sources for lipase production from *Penicillium verrucosumin* [47]. In order to improve the productivity of lipase from *Rhizopus chinesis*, the effect of oils and oil-related substrates were assessed by orthogonal test and Response Surface Methodology (RSM) [98]. The optimized medium for improved lipase activity consisted of peptone, olive oil, maltose, K2HPO4, and $MgSO_4.7H_2O$ [98]. The Plackett–Burman statistical experimental design was used to evaluate the fermentation medium components [46]. The effect of 12 medium components was studied in 16 experimental trials. Glucose, olive oil, and peptone were found to have more significant influence on lipase production by *Candida rugosa*. RSM approach was used to investigate the production of an extracellular lipase from *Aspergillus carneus*. Interactions were evaluated for five different variables (sunflower oil, glucose, peptone, agitation rate, and incubation period) and 1.8-fold increase in production was reported under optimized conditions [44]. Lipase production was observed in the range of 7.78 U/ml to 6,230 U/ml under various optimized conditions [32, 99, 100].

Purification

Many fungal lipases have been extensively purified and characterized in terms of their activity and stability profiles at different pH, temperature, effects of metal ions, and chelating agents. Purification of enzymes allows determination of primary amino acid sequence and 3-D structure ([101-103], and X-ray studies of pure lipases enable the establishment of the structure–function relationships and contribute for a better understanding of the kinetic mechanisms of lipase action on hydrolysis, synthesis and group exchange of esters [17]. The purification of lipase from different microorganisms has been reported through several techniques such as precipitation ([102, 104], hydrophobic interaction chromatography [105], gel filtration [106], ion exchange chromatography [107], and affinity chromatography [108, 109]. Purification of lipase is needed in industries employing the enzymes for the biocatalytic production of fine chemicals [110], pharmaceuticals [111], and cosmetics [112]. During the early stages of a purification method, precipitation was used as a crude separation step and was found to give a high average yield [102].

A lipase from *Penicillium cyclopium* MI was purified by using ammonium sulfate precipitation, DEAE Cellulose, DEAE-Sepharose, hydroxyapatite chromatography, and gel filtration on Cellulofine GC-700. The purification of the preparation was 1,380-fold and recovery yield 27%. The molecular weight of the enzyme was estimated to be 35,000 g/mol from Sephadex G-100 chromatography [40]. The lipase obtained from Trichoderma viride was

purified 134-folds with 46% yield by ion exchange and gel permeation chromatography [113]. A novel thermostable lipase from *Aspergillus niger* was purified from a crude preparation by a procedure including precipitation followed by a series of chromatographic steps. The overall purification was 50-fold with a yield of 10% [114]. The lipase from *Rhizopus japonicus* NR400 was purified to homogeneity by chromatography on hydroxyapatite, octylsepharose, and Sephacryl S-200 [17]. Microbial lipases showed different molecular weights ranging between 25-68 kDa [115-122]. The highest molecular weight of lipase i.e. 70 kDa has been reported from thermophilic fungus *Neosartorya fischeri* P1 [123].

Immobilization of Lipases

Immobilization improves recyclability of expensive lipases and also enhances enzyme stability and activity. Immobilization is favored as it can easily control the enzymatic process, purity of the products, and for its reusability feature [124, 125]. Using immobilized lipases has multi-fold advantages such as increase in thermal and ionic stability which also increases its efficiency. It is also easier to control reaction parameters like flow rate and accessibility of substrates when the enzyme is immobilized [126, 127]. The major contribution to achieve a good performance of immobilized catalyst is primarily provided by the strategy employed for immobilization [128] and by the characteristics of the support. The desirable characteristics of solid supports used for immobilization include large surface area, low cost, reusability, good chemical, mechanical and thermal stability, and insolubility [129].

Different techniques for immobilization of lipases, such as physical adsorption, covalent bonding, entrapment and microencapsulation using various supports have been used [130-139].

Best support for immobilization of lipase from *A. niger* was found to be Amberlite MB-1, which gave an immobilization yield of approximately 62% [140]. The immobilization of purified lipases obtained from a commercial *A. niger*, via ionic adsorption on DEAE-Sepharose was reported by [141]. The immobilization of *Aspergillus sp*. lipase in silk fibers via glutaraldehyde cross-linking, and its use in hydrolysis of sunflower oil has been reported [142]. *Candida rugosa* lipase immobilized on oxidized multi-walled carbon nanotubes (MWCNTs) resulted in enhancement in catalytic activity of the enzyme [143]. Extracellular lipase from *Yarrowia lipolytica* IMUFRJ 50682, when immobilized on nano-sized magnetic particles, pH and thermostability of the enzyme increased [144].

Lipase from *Candida antarctica* (CALB) immobilized in gigaporous PGMA microspheres showed the highest activity yield, reusability, stability, as well as the best affinity for the substrate [3]. Hydrophobic controlled pore glasses were employed to immobilize *Rhizomucor miehei* lipase [145]. In a recent study, lipase from *Trametes hirsute* was immobilized on chitosan/clay beads, with an immobilization yield of 80.9%. The analysis of free enzyme and the immobilized derivative at different temperatures, pH, in the presence of various solvents, metallic ions, and storage showed that the immobilization process increased the enzyme life span [146]. A method has been reported for covalent attachment of *Candida rugosa* lipase to two types of chitosan beads by activating the hydroxyl groups using carbodiimide as the coupling agent. Immobilization enhanced the enzyme stability against changes in pH and temperature, and increased enzyme activity up to 110%. Lipase from *Candida rugosa* was found to be more stable when entrapped in alginate gel than covalently bound on Eupergit C or encapsulated in a sol-gel matrix [147]. *Yarrowia lipolytica* lipase was immobilized on octyl-agarose and octadecyl-sepabeads supports by physical adsorption that resulted in higher yields and greater (10-fold) stability than that of free lipase. This was accounted by the hydrophobicity of octadecyl - sepabeads that enhanced affinity

Table 2. Lipase applications in the food industry [18].

Food industry	Action	Product of application
Dairy foods	Hydrolysis of milk fat, cheese ripening, modification of butter fat	Development of flavoring agents in milk, cheese and butter
Bakery foods	Flavor improvement	Shelf-life extension, volume improvement
Beverages	Improved aroma	Alcoholic beverages, e.g. sake, wine
Food dressings	Quality improvement	Mayonnaise, dressings and whippings
Health foods	Transesterification	Health foods
Meat and fish	Flavor development	Meat and fish product, fat removal
Fats and oils	Transesterification, hydrolysis	Cocoa butter, margarine, fatty acids, glycerol, mono and diglycerides

between the enzyme and support [148]. Lipases entrapped in k-carrageenan have been reported to be highly thermostable and organic solvent tolerant [149, 150].

Applications

Fungal lipases are widely diversified in their enzymatic properties and substrate specificity, which makes them very attractive for industrial applications. They constitute an important group of biotechnologically important enzymes because of the versatility of their properties and ease of mass production. The industrial applications of fungal lipases have been reviewed by many researchers [1, 2, 151-153]. Development of lipase-based technologies for the synthesis of novel compounds is rapidly expanding the uses of these enzymes [154].

Lipases in food processing industry

Fats and oils are important constituents of foods and their modification is one of the prime areas in food processing industry that demands novel economic and green technologies [155]. Most of the commercial lipases produced are utilized for flavor development in dairy products and processing of other foods, such as meat, vegetables, fruit, baked foods, milk products, and beer [156, 157]. Lipases from *A. niger*, *Rhizopus oryzae*, *Candida cylindracea* have been used in bakery products. Betapol was the first commercial product made by the 1,3-specific lipase treatment of tripalmitin with unsaturated fatty acids that resulted in 1,3-diunsaturated-2-saturated triglycerides intended for infant formula [158, 159]. Immobilized lipases from *Candida antarctica* (CAL-B), *Candida cylindracea* AY30, and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil [160]. A whole range of microbial lipase preparations such as *Mucor meihei* (Piccnate, Gist-Brocades; Palatase M, Novo Nordisk), *A. niger* and *A. oryzae* (Palatase A, Novo Nordisk; Lipase AP, Amano; Flavour AGE, Chr. Hansen) have been developed for the cheese manufacturing industry (http://www.au-kbc.org/frameresearch.html). Lipase synthesized from *Penicillium roquefortii* is largely responsible for the development of the characteristic flavor of blue cheese [161, 162]. In recent years, consumers have been increasingly confronted with functional foods and nutraceuticals, which are claimed to promote health and wellbeing beyond their nutritive properties ([163, 164]. Large scale applications of lipases in industry can be found not only in the dairy and baking industry but also for the production of trans-fatty acid free margarines [74, 165, 166]. Lipase applications in various food industries are given in Table 2 [18].

Lipase as biosensor

In clinical diagnosis and in food industry, the quantitative determination of triacylglycerol is of great importance. The lipid sensing device as a biosensor is rather cheaper and less time consuming as compared to the chemical

methods for the determination of tryacylglycerols. The basic concept of using lipase as biosensors is to generate glycerol from the triacylglycerol in the analytical sample and to quantify the released glycerol by an enzymatic method [151]. Lipases immobilized on pH/oxygen electrodes along with glucose oxidase serve as lipid biosensors and can be used for the determination of triglycerides and blood cholesterol [167]. Lipase biosensor is also used for the determination of lipids for the clinical diagnosis [168]. Lipase from *Candida rugosa* has been developed as a DNA probe [110]. The enzyme lipase immobilized in a Nafion membrane on a graphite-epoxy transducer can be used to quantify triglycerides in food samples [169]. *Candida rugosa* lipase was immobilized on aluminosilicate and used for the detection of an organo phosphate insecticide (Diazinon) in an aqueous medium [170]. In another study, a *Candida rugosa* lipase immobilized on a mesoporous Si matrix was used for the detection of triglycerides [171]. Lipase was also used as a amperometric sensor [172]. *Candida rugosa* lipase, acts as a catalyst in the hydrolysis of triacylglycerol to glycerol and fatty acids, is used as biosensors for detection of β-hydroxyacid esters and triglycerides in blood serum [173].

Lipases in ester synthesis

Lipases have been used for the synthesis of esters. The esters produced from short chain fatty acids have applications as flavoring agents in food industry [174]. Various esterification reactions catalyzed by lipases are shown in Table 3. Lipase from *Bacillus aerius* immobilized on celite 545 was used for the synthesis of ethyl ferulate, a compound used for anticancer properties [185]. As reported earlier, esterification of sulcatol and fatty acids in toluene was catalyzed by *Candida rugosa* lipase [186]. The esterification reaction of lauryl alcohol and palmitic acid with *C. Antarctica* lipase (Novozym 435) as the catalyst has been reported to give a yield of more than 90% of lauryl palmitate under optimized conditions [187]. Lipase immobilized on silica and microemulsion- based organogels has been used for ester synthesis [188]. In a recent study, lipase from *Aspergillus ibericus* has been used for the esterification reactions and aroma ester production [189]. A variety of fatty acid esters are now produced commercially by using immobilized lipase in nonaqueous solvents [190-193].

Lipases in bioremediation

Bioremediation for waste disposal is a new avenue in lipase biotechnology. Lipases have been extensively used in waste water treatment [194]. Fungal species can be used to degrade oil spills in the coastal environment, which may enhance ecorestoration as well as help in the enzymatic oil processing in industries [195]. Species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Mortierella*, *Beauveria,* and *Engyodontium* are some examples of the fungi that have recently been described as tolerant to a variety of pollutants and indicated as potential bioremediation agents in soil [196]. Lipase from *Aspergillus niger* and *Aspergillus terreus* were used for the degradation of polyvinyl alcohol films and bioremediation of polluted soils respectively [197, 198]. Lipase from *Aspergillus ibericus* and *Aspergillus uvarum* were also used in bioremediation processes [199]. Lipolytic enzyme obtained from *Aspergillus niger* isolated from oil polluted soil has been examined and found to degrade polyaromatic hydrocarbons found in petroleum contaminated soil [81].

Lipases in textile industry

The use of fungal lipase in textile industry is becoming increasingly important. Lipases are used to assist in the removal of size lubricants in order to provide the fabric better absorbency for enhanced levelness in dyeing. Commercial preparations used for the desizing of denim and other cotton fabrics contain lipase enzymes [151]. Lipases together with alpha amylase are being used for the desizing of the denim and other cotton fabrics at the commercial scale [200]. *Aspergillus oryzae* lipase was capable of modifying PET (Polyethylene terephthalate) fabrics, improving their hydrophilicity and anti-

Table 3. Esterification catalyzed by lipases.

Lipase	Acid	Alcohol	Solvent	Reference
Rhizopus delemar *Penicillium roqueforti* *Humicola lanuginosa*	C_4	C_2, C_4, Isoamyl	Hexane	[175]
Mucor miehei	C_{12}, Oleic	C_3-C_{12}	-	[176]
Geotrichum candidum *Aspergillus niger* *Rhizopus delemar* *Penicillium cyclopium*	Oleic	Terpene alcohol, primary alcohols (C_1-C_{12}), 2- and 3-substituted alcohols, benzyl alcohol, cyclohexanol	Buffer+Casein	[177]
Aspergillus niger Rhizopus *delemar* *Penicillium cyclopium* *Geotrichum candidum*	C_2-C_{18}, benzoic, oleic, ricinoleic, sebacic, succinic etc.	Glycerol	Water	[178]
Aspergillus niger *Rhizopus delemar* *Penicillium cyclopium*	C_3-C_6, Isobutyric	Geraniol, farnesol, phytol, β-citronellol	-	[179]
Mucor miehei	C_4	C_4	Hexane	[180]
Candida rugosa	Oleic	Sucrose, sorbitol, glucose, fructose	Buffer (pH 5.4)	[181]
Candida rugosa	Oleic, isostearic, 12-hydroxystearic, stearic	Cholesterol	Cyclohexane	[182]
Candida antartica	Melted coconut acids	Ethyl D-glucopyranoside	-	[183]
Mucor miehei	Oleic, linoleic, α-linoleic, γ-linoleic. Docosahexanoic	C_2	Pentane	[184]

static ability [201]. Immobilization of lipase from porcine-pancreas onto zirconia coated alkylamine glass beads by glutaraldehyde coupling was carried out for better washing of cotton cloth [202].

Lipases in detergent industry

Fungal lipases find a major use as additives in detergents for industrial laundry and household detergents [203], and this can reduce the environmental load of detergent products, as it saves energy by enabling a lower wash temperature to be used [204]. An estimated 1,000 tons of lipases are added to the approximately 13 billion tons of detergents produced each year. In 1994, Novo Nordisk introduced the first commercial lipase, Lipolase™, which originated from the fungus *Thermomyces lanuginosus* and was expressed in *Aspergillus oryzae*. Lipase from *Thermomyces sp*. is the most important detergent lipase which is very commonly used (Lipolase, Novozymes) [205]. A novel thermoactive and alkaline lipase from *Talaromyces thermophilus* fungus showed great resistance to alkaline pH, interfacial denaturation, and a high tolerance to various surfactants, oxidizing, and commercial wash agents. This enzyme could therefore be considered as a satisfactory and promising candidate for further industrial application principally cleaning process [206]. Lipase of *Humicola lanuginosa* is suitable as a detergent additive because of its thermostability, high activity at alkaline pH, and stability towards anionic surfactants. Lipases used as detergents also include those from *Candida* [207]. Laundering is generally carried out in alkaline media, lipases active under such conditions are preferred [208-210], for example, the *A. oryzae* derived lipase. The other applications of detergents are in dish washing, in a bleaching composition [211], decomposition of lipid

contaminants in dry cleaning solvents [212], liquid leather cleaner [213], contact lens cleaning [214], washing, degreasing, and water reconditioning by using lipases along with oxidoreductases, which allows for smaller amounts of surfactants and operation at low temperatures [215]. The lipase component causes an increase in detergency and prevents scaling. Recently, lipase from *Rhizopus nigricans* showed maximum lipolytic activity as well as bioemulsification activity indicating highest biosurfactant production also [216].

Lipases in medical applications

Lipases are evolving rapidly and currently they are reported to show high potential in medicine. Intensive study and investigations have led researchers to explore lipases for their use in substitution therapy, where in enzyme deficiency during diseased conditions is compensated by their external administration [9]. Lipases may be used as digestive aids [174, 208] and as the activators of Tumor Necrosis Factor, and therefore, can be used in the treatment of malignant tumors [217]. Although human gastric lipase (HGL) is the most stable acid lipase and constitutes a good candidate tool for enzyme substitution therapy [218]. Lipases have earlier been used as therapeutics in the treatment of gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. [219]. Lipase from *Candida rugosa* immobilized on a nylon support has been used to synthesize lovastatin, a drug which lowers serum cholesterol levels [220].

Lipases in Paper Industry

Lipolytic enzymes are used to remove pitch, the lipid fraction of wood that interferes with the elaboration of paper pulp. They also help in the removal of lipid stains during paper recycling and to avoid the formation of sticky materials [151, 221]. Nippon Paper Industries in Japan developed a pitch control method that used a fungal lipase from *Candida rugosa* to hydrolyse up to 90% of the triglycerides [18]. Hata and coworkers at Jujo Paper Company reported in 1990 that lipases could reduce pitch problems by lowering the triglyceride content of groundwood pulp. A lipase obtained from *Candida cylindrica*, when added to the groundwood stock chest, reduced pitch problems and talc consumption considerably. *Candia antartica* lipase A (CALA) was used in pitch control in the paper industry [222].

Lipases in cosmetics and personal care products

The cosmetic sector lipases have been used for personal care such as cleaning, softening, aroma, and coloring. It has large market value after food and pharma sector and accounts for 200 billion Euro [223]. Lipases have potential application in cosmetics and perfumeries because they show activities in surfactants and in aroma production [112]. Transesterification of 3,7-dimethyl-4,7-octadien-1-ol with lipases from various microbial sources has been done to prepare rose oxide, which is an important fragrance ingredient in the perfume industry [224]. Nippon Oil and Fats also obtained a patent for the preparation of propyleneglycerol monofatty acid ester in the presence of lipase. This ester has been used as emulsifier and a pearling agent in cosmetics and foods [225]. Lipases are used in hair waving preparation [226] and have also been used as ingredients of topical antiobese creams [227] or in oral administration [228]. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase [229]. Non-specific lipase derived from *Candida antartica*, marketed as Novozym 435, was determined to be the most suitable for the enzymatic synthesis of isopropyl myristate [230]. Immobilized *Rhizomucor meihei* lipase was used as a biocatalyst in personal care products such as skin and sun-tan creams, bath oils etc. *Candida antarctica* lipase B synthesized amphiphilic compounds receive great attention from cosmetic industry due to a range of beneficial properties for skin [231].

Lipases in biodiesel production

Biodiesel is a group of esters produced by transesterification reaction between fatty acids and an alcohol in presence of catalyst. The biodiesel production from waste and non-edible

vegetable oil greatly reduces the cost of biodiesel production, and thus avoids the conflict between food and energy security, and is considered an important step in reducing pollution and recycling waste oil [10, 232].

The production of biodiesel has risen sharply in the last decade from approximately 950 liters in 2000 to nearly 17,000 million liters in 2010 with the European Union as the world's major producer, accounting for 53% of global biodiesel production [233]. In 2000, Biodiesel represented around 5% of the world's biofuel production and in 2011 biodiesel share accounted for around 20% of total biofuel production [233]. This increase seems to continue and biodiesel production is estimated to reach 41,000 million liters in 2022, as reported by the United Nations. Higher thermostability and short-chain alcohol-tolerant capabilities of lipase make it very convenient for use in biodiesel production [234, 235]. The majority of yeast and fungal lipases involved in biodiesel production are *A. niger*, *C. antartica*, *C. rugosa*, *R. miehei*, *R. oryzae*, and *Thermomyces lanuginose* [236].

Production of biodiesel has been reported by using immobilized *Candida antarctica* lipase-catalyzed methanolysis of soybean oil [237]. Immobilized lipase from *Candida rugosa* on Sepabeads EC-OD was most promising as a biocatalyst for the application of enzyme-catalyzed biodiesel synthesis [238]. In a recent study, biodiesel production from Chinese tallow kernel oil has been catalyzed by *Candida rugosa* lipase (CRL) in ionic liquid [239].

Lipases in leather industry

In recent years, lipases have found application in the soaking, dehairing, bating, and degreasing operation in leather making. Hides and skins contain proteins and fat in the collagen fibers. These substances must be partially or totally removed before the hides and skins are tanned. Lipases specifically degrade fat and do not damage the leather itself. Lipases represent the method of removing fat in the degreasing process with the lowest environmental impact [240]. For bovine hides, lipases allow tensile to be completely replaced. For sheepskins, the use of solvents is very common, but it can also be replaced by lipases and surfactants.

Conclusion

Fungi are capable of producing several enzymes for their survival within a wide range of substrates. Among these enzymes, lipases are predominantly used in several applications. Lipases owing to their properties such as activity over a wide temperature and pH range, substrate specificity, diverse substrate range and enantio-selectivity are the biocatalysts of choice for the present and future. The growing demand for lipases has shifted the trend towards prospecting for novel lipases, improving the properties of existing lipases for established technical applications and producing new enzymes for new areas of application. They are one of the most versatile enzymes available in nature. They are unique in various aspects starting from their ability to act at the interface, to molecular imprinting and retention of activity in organic solvents. These fat-splitting enzymes are attractive because of their applications in fields relevant to food, textile, biodiesel, medicine, paper, dairy, detergent and leather industry. The tremendous potential of lipases in various industries shows the need to develop novel cost-effective technologies for increased production, scaling up and purification of this versatile enzyme.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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