



Chapter 1

Lipases: An Overview

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Abstract

Lipases are ubiquitous enzymes, widespread in nature. They were first isolated from bacteria in the early nineteenth century, and the associated research continuously increased due to the characteristics of these enzymes. This chapter reviews the main sources, structural properties, and industrial applications of these highly studied enzymes.

Key words Lipases, Structure, α/β hydrolase fold, Lid, Oxyanion hole, Catalytic triad, Catalytic mechanism, Selectivity, Applications

1 Definition of Lipases

Lipases are serine hydrolases defined as triacylglycerol acylhydrolases (E.C. 3.1.1.3) and should be differentiated from esterases (E.C. 3.1.1.1) by the nature of their substrate. Indeed, a criteria used to distinguish these two types of enzymes, i.e., activation by the presence of an interface, also called “interfacial activation,” was found unsuitable for the classification of such enzymes as some lipases did not exhibit such phenomenon. Prominent cases of this phenomenon are Lip4 from *Candida rugosa* [1] and *Candida antarctica* B [2]. Moreover, lipase and esterase consensus motifs described by PROSITE database [3] are very close. Therefore, lipases were later defined as enzymes capable of hydrolyzing carboxyl esters of long-chain acylglycerol (≥ 10 carbon atoms), while esterases hydrolyze carboxyl esters of short-chain acylglycerol (≤ 10 carbon atoms). Nevertheless, as both enzymes show a broad substrate specificity, both criteria should be considered [4–6]. Fojan et al. also proposed a novel approach to distinguish between esterases and lipases based on the study of the amino acid composition and protein surface electrostatic distribution [7]. Further research on lipases and esterases showed that in the hydrolysis of soluble esters, lipase K_m is higher than the K_m of esterases [5].

In addition, lipases are capable of hydrolyzing water-insoluble esters and show a different distribution on the hydrophobic amino acids surrounding the active site [5]. Cutinases usually catalyze the hydrolysis of ester bonds in cutin polymers; however they are also capable of hydrolyzing long-chain and short-chain triglyceride without requirement of interfacial activation; therefore they have characteristic similar to lipases and esterases [8]. This last one will not be discussed in this review.

2 Reaction Catalyzed by Lipases

2.1 Hydrolysis Reaction

Lipases naturally catalyze the hydrolysis of the ester bond of tri-, di-, and monoglycerides into fatty acids and glycerol (Fig. 1). Nevertheless, as shown in Fig. 1, they are also active on a broad range of substrates. In all cases, the reaction is carried out at the interface of a biphasic system reaction. This biphasic system results from the presence of an immiscible organic phase, containing the hydrophobic substrate, in water.

2.2 Synthesis Reactions

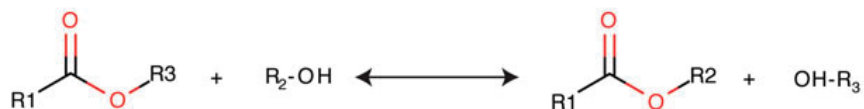
Lipases, in thermodynamic favorable conditions (i.e., low water activity), also catalyze a large variety of synthesis reactions which can be classified into two main types of reactions, i.e., esterification and transesterification [9]. As shown in Fig. 1, esterification is the reaction where a fatty acid is linked, through the action of the enzyme, to an alcohol by a covalent bond, producing an ester and releasing a water molecule. Thioesterification and amidation are similar reactions but with a thiol or an amine as substrates. Transesterification groups alcoholysis, acidolysis, aminolysis, and interesterification reactions.

Usually, these synthesis reactions occur in a medium with low thermodynamic water activity, the thermodynamic activity being a measure of the molecule availability in a solvent. The medium then consists in a free solvent system, in molten medium, or in an organic solvent.

Lipases are also capable of expressing other related activities such as phospholipase, lysophospholipase, cholesterol esterase, cutinase, or amidase activities [10]. Furthermore, lipases can catalyze Michael addition [11] and epoxidation reactions [12].

3 Sources of Lipases and Its Physiologic Role

First lipases were isolated by Eijkman from *Bacillus prodigiosus*, *Bacillus pyocyaneus*, and *Bacillus fluorescens*, currently known as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*, respectively [13]. Nowadays, it is recognized that lipases are produced by various organisms, including animals, plants, and

Hydrolysis**Synthesis***Esterification**Esterification**Amidation**Thioesterification***Transesterification***Acidolysis reactions**Aminolysis reaction**Alcoholysis reaction**Interesterification reaction***Fig. 1** Reactions catalyzed by lipases

microorganisms. Most animal lipases are obtained from the pancreas of cattle, sheep, hogs, and pigs. Unfortunately, lipases extracted from animal pancreas are rarely pure enough to be used

in the food industry. For example, pig pancreatic lipase is polluted by trace amounts of trypsin which generate a bitter taste [14]. Other impurities include animal viruses and hormones. Therefore, due to the ease of production and abundance, most studied and industrially used lipases are obtained from microbial sources. Moreover, compared to bacterial lipases, lipases from GRAS (generally recognized as safe) yeast sources are widely accepted and used in several industries including food processing [15]. Some of the major lipases used in industrial processes are discussed in Subheading 6.

Due to the importance and wide variety of lipase applications, different techniques have been developed in order to isolate lipases from various sources. Due to their ability to use fat as the only carbon source, microorganisms producing lipases have been isolated from food spoilage, where they are responsible for the flavor change of dairy products such as cheese, or from oily environments (sewage, rubbish dump sites, and oil mill effluent). For instance, a cold-adapted lipase was isolated from *Pseudomonas stutzeri* PS59 by screening soil samples from contaminated areas in olive oil-enriched medium [16]. With a similar method, an alkali-thermostable lipase from *Staphylococcus aureus* was isolated from agricultural fields by inoculation into a plate with nutrient agar and tributyrin [17]. New lipases have also been successfully isolated from contaminated butter [18], algae from an Antarctica lake [19], activated sludge, wastewater, and petroleum-contaminated soil samples [20], among others. Nevertheless, this direct method cannot be applied to uncultivable organisms and do not allow the isolation of the gene encoding the lipase.

New methods have been developed to allow the discovery of enzyme genes. These metagenomic approaches are applicable to uncultivable organisms [21]. These include the screening of DNA libraries, created from lipase-producing microorganisms by PCR with degenerate oligonucleotides complementary to lipase gene-conserved regions as probe [22]. An alternative to this method is functional metagenomic, i.e., the cloning of a so-called metagenome isolated from environmental DNA and its expression in a host for further screening of its hydrolytic activity [6]. An increasing number of methods have been developed to allow the detection of lipolytic activities [23].

Lipases are known to have several physiological functions. In eukaryotes, they are key components of lipid and lipoprotein metabolism [24]. As so, they are produced in the digestive system to hydrolyze absorbed triglycerides. Their production would be activated by a hormone-sensitive regulation system when the energy demand increases, thus initiating the degradation of reserve triglycerides. In insects, lipases are used for the digestion of dietary fats, hydrolysis of lipids, and lipid mobilization [25]. In plants, lipases are mainly located in seeds, as part of the energy reserve

tissues, and carry out the hydrolysis of reserve triglycerides necessary for postgerminative growth [26]. In addition, they play a defensive role since their production is induced upon wounding [27, 28]. Microorganisms use the production of extracellular lipases in order to hydrolyze the triglycerides in the media and facilitate the ingestion of lipids.

Lipase expression in microorganisms is mainly regulated by environmental factors, as an extracellular response to a medium deprived in nutrients. In most microorganisms, the presence of lipids and fatty acids as carbon sources induces the production of these extracellular enzymes, thus allowing them to grow on spoiled soil. Microorganisms are the most interesting host for recombinant protein production, where regulated or constitutive promoters can be used for the fermentation process [29].

4 Structure and Catalytic Mechanism

The first lipase structures were obtained from *Rhizomucor miehei* [30] and the pancreatic human lipase [31]. Several hundreds of lipase sequences are listed in databases, in 2012 the Protein Data Base (<http://www.rcsb.org/pdb/home/>) had among 100 three-dimensional lipase structures [32]; nowadays this database has 182 three-dimensional lipase structures. However, these 182 structures represent lipases of only 49 organisms, since the same lipase can have several structures in different conformations or with different substrates. These include fungal lipases such as those from *Thermomyces lanuginosus* [33], *Rhizopus oryzae* and *niveus* [33, 34], *C. antarctica* lipase B and lipase A [35, 36], *C. rugosa* [37], *Geotrichum candidum* [38], *Penicillium camemberti* and *expansum* [39, 40], *Yarrowia lipolytica* [41], *Saccharomyces cerevisiae* [42, 43], *Gibberella zeae* [44], *Malassezia globosa* [45, 46], and *Penicillium cyclopium* [47]. As well, the known structures of bacterial lipases include those from *Bacillus* sp. [48], *B. subtilis* [49], *Pseudomonas* sp. [50], *P. aeruginosa* [51], *B. cepacia* [52, 53], *B. glumae* [54], *Chromobacterium viscosum* [55], *Geobacillus thermocatenulatus* [56], *Geobacillus stearothermophilus* [57, 58], *G. zalihiae* [59], *Photobacterium* sp. [60], *S. marcescens* [61], *Staphylococcus hyicus* [62], *Streptomyces exfoliatus* [63], *Proteus mirabilis* [64], and *Pelosinus fermentans* [65]. In addition, the structures of *Archaeoglobus fulgidus* lipase [66], bovine bile lipase [67], and dog [68], horse [69], rat [70], and guinea pig [71] pancreatic lipase have been obtained. Lipases from *C. rugosa*, *C. antarctica*, *P. aeruginosa*, *T. lanuginosus*, and *C. viscosum* have a wide variety of industrial applications.

Structurally speaking, lipases are characterized by a common α/β hydrolase fold and a conserved catalytic triad. Most lipases also possess the consensus motif G-X1-S-X 2-G. From their structures and the residues forming the oxyanion hole (amino acids of the lipase

active site that stabilize the reaction intermediate) and catalytic triad, microbial lipases, esterases, and related proteins can be grouped into 38 superfamilies and 112 homologous families (Lipase Engineering Database, <http://www.led.uni-stuttgart.de>). More recently the ESTHER database classified the superfamily of α/β hydrolases into 93 families and presents the correspondence between the ESTHER and Lipase Engineering Database families [72]. The lipase structural elements are discussed below. Table 1 gives a summary of the structural data available for some extensively studied lipases.

4.1 The α/β Hydrolase Fold

The study of lipase three-dimensional structures showed the presence of a conserved alpha/beta-hydrolase fold, which is widely expanded in hydrolytic enzymes of different origins, such as haloalkane dehalogenases, acetylcholinesterases, diene lactone hydrolases, serine carboxypeptidases, carboxylesterases, cutinases, thioesterase, proline iminopeptidase, proline oligopeptidase, and epoxide hydrolase [96, 72]. The alpha/beta-hydrolase fold is generally composed of a central, parallel β -sheet of eight beta-strands, with only the second strand antiparallel (β_2) and ordered as 12435678 [72]. Strands β_3 – β_8 are connected by α -helices arranged on the sides of the central β -sheet [96] (Fig. 2).

Some variations of the α/β fold are found in several lipases. The variations of the fold consist in differences in the amount of α -helices, β -sheets, curvature of the β -sheet, loops length, and architecture of the substrate binding sites [49, 96, 97].

Lipases are also characterized by the presence of disulfide bridges that give the enzyme stability and are often important for their catalytic activity [98, 99].

4.2 The Catalytic Triad

The catalytic triad, which is conserved among lipases, consists of a serine as nucleophile, an aspartate/glutamate as the acidic residue, and a histidine [9, 72]. It is similar to the one observed in serine proteases but with a different order in the sequence [100]. In the alpha/beta-hydrolase fold, the catalytic serine is located in a γ -like turn, after the sheet- β_5 and before the following α -helix, in a highly conserved structural feature of the fold [72]. The aspartate or glutamate is found in a loop after the β_7 -sheet, and the histidine is located in a loop after the β_8 -sheet [101]. Another subclass of esterase/lipase has been reported, in which the G-X1-S-X2-G consensus sequence containing the catalytic serine is replaced by a GDSL sequence located close to the protein N-terminus [102].

4.3 The Oxyanion Hole

The tetrahedral intermediate formed during the catalytic mechanism of lipases is stabilized by the presence of hydrogen bonds with two amino acids that form the so-called lipase oxyanion hole. These amino acids stabilize the intermediate through hydrogen bonds between their backbone amide proton and the oxygen of the substrate carbonyl group (*see* Subheading 4.6) [103–105].

Table 1
Summary of the structural data available for some extensively studied lipases

Lipase	PDB entry	Catalytic triad	Oxyanion hole	Lid	References
<i>Burkholderia cepacia</i>	1OIL 2LIP 3LIP 4LIP 5LIP 1HQD 1YS1 1YS2 2NW6	S87, D264, H286	L1, Q88	E118- T150	[52] [53] [73] [74] [75] [76]
<i>Candida antarctica</i> B	1TCA 1TCB 1TCC 1LBS 1LBT 3ICV 3ICW 4K5Q 4K6G 4K6H 4K6K 5A6V 5A71 4ZV7	S105, D187, H224	T40, Q106	–	[36] [77] [78] [79] [80] [81]
<i>Candida rugosa</i>	1CRL 1TRH 1LPN 1LPO 1LPP 1LPM 1LPS 1GZ7 3RAR	S209, E341, H449	G124, A210	E66- P92	[37] [82] [83] [84] [85] [86]
<i>Rhizomucor miehei</i>	1TGL 3TGL 4TGL 5TGL	S144, D203, H257	S82, L145	S83-P96	[30] [87] [88] [89]
<i>Rhizopus oryzae</i>	1TIC	S145, D204, H257	T83, L146	N84- F95	[33]
<i>Thermomyces lanuginosus</i>	1TIB 1DT3 1DT5 1DTE 1DU4 1EIN 1GT6 4ZGB 5AP9	S146, D201, H258	S83, L147	R84- F95	[33] [90] [91] [92] [93]
<i>Yarrowia lipolytica</i> (lipase 2)	3O0D	S162, D230, H289	T88, L163	T88- L105	[41]
<i>Saccharomyces cerevisiae</i> (Lpx1)	2Y6U 2Y6V	S145, E169, His323	–	T174- P190	[43]
Human pancreatic lipase	1N8S 2PVS 2OXE	S153, H264, D177	F78, L154	–	[94] [95]

The first residue of the oxyanion hole is the X2 residue of the consensus sequence G-X1-S-X2-G, located after strand β 5, next to the catalytic serine, in the structurally conserved nucleophilic elbow common to all lipases [105]. The second residue of the oxyanion

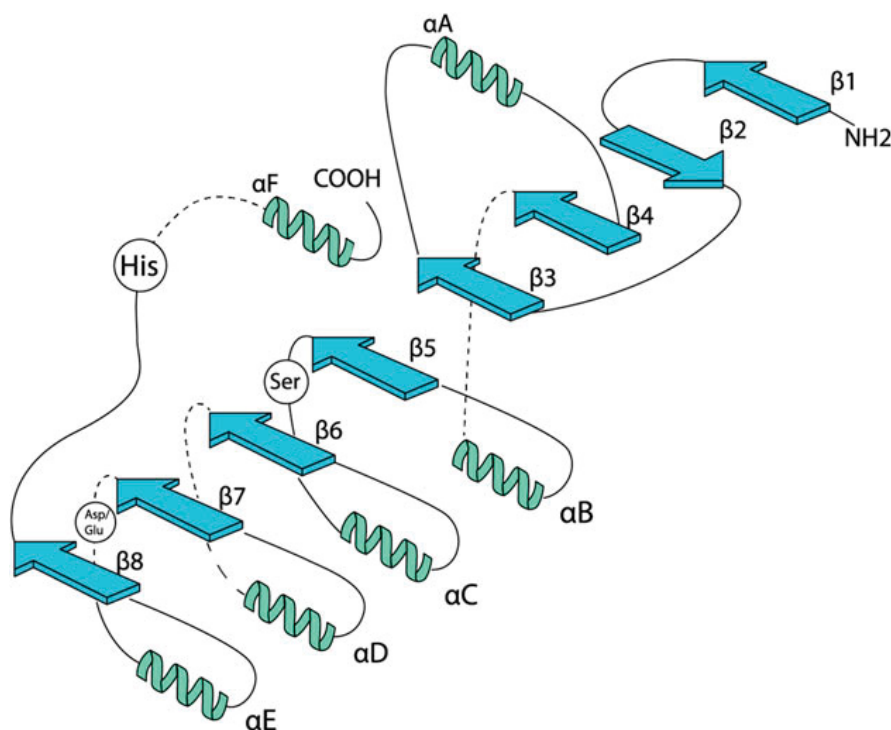


Fig. 2 The alpha/beta-hydrolase fold; α -helices are represented by spirals and β strands are indicated by arrows. The residues of the catalytic triad are shown in circles (Adapted from [96])

hole is located in the N-terminal part of lipases, in the loop between the $\beta 3$ -strand and the αA -helix [103]. From the Lipase Engineering Database, lipases are classified on the basis of their oxyanion hole into three classes: *GX*, *GGGX*, and Υ . Pleiss et al. [105] identified two types of oxyanion holes, *GX* and *GGGX*, which are shown in Fig. 3. In the *GX* type, *G* is a conserved glycine, and *X* is the oxyanion residue. In the *GGGX* type, the oxyanion hole residue **G** is followed by a conserved hydrophobic residue *X*. The oxyanion hole can either be preformed in the closed conformation [64] without the geometrical modification upon the opening of the lid or only formed upon opening of the lid [106] (see Subheading 4.4).

The type of oxyanion hole plays an important role in the specificities of lipases toward their substrates. Indeed, lipases with the *GX* type usually hydrolyze substrates with medium and long carbon chain length, while the *GGGX* type is found in short length-specific lipases and carboxylesterases. Fungal lipases have the oxyanion hole type *GX*, where *X* is either a serine or threonine, and in most cases they possess a third amino acid, aspartic or asparagine, which also contributes to stabilize the oxyanion hole through a hydrogen bond [105].

A third type of oxyanion hole, type Υ , was identified by Fischer et al. [107]. In type Υ the oxyanion hole is formed by the hydroxyl group of a strictly conserved tyrosine side chain. This type is found in lipase A from *C. antarctica* (family abh38); few esterase, such as cocaine esterases; and some bacterial lipases [107–109].

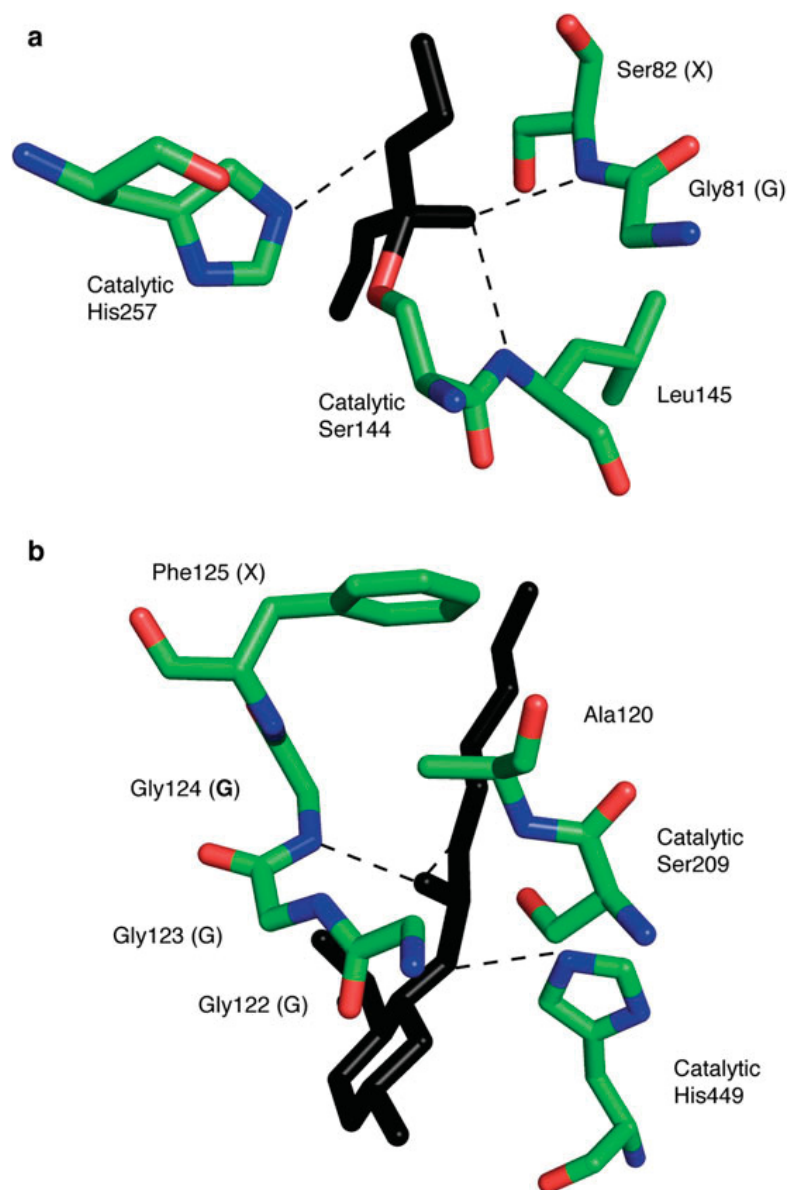


Fig. 3 Two types of oxyanion holes. (a) GX type in *R. miehei* lipase (PDB entry 4TGL): diethyl phosphonate stabilized by hydrogen bonds with Ser82 and Leu145. (b) GGGX type in *C. rugosa* lipase (PDB entry 1LPM): (1R)-menthyl hexyl phosphonate stabilized by hydrogen bonds with Gly124 and Ala210. Substrate is shown in black and hydrogen bonds are schematized by dotted lines

GDSL enzymes do not have the so-called nucleophilic elbow, and their oxyanion hole seems to have a particular structure: the catalytic Ser, located close to the N-terminus, serves as a proton donor in the oxyanion hole, together with a highly conserved glycine and asparagine [110]. This tri-residue-constituted oxyanion hole was proposed to compensate for the lack of hydrogen bond of the intermediate with the catalytic histidine [111].

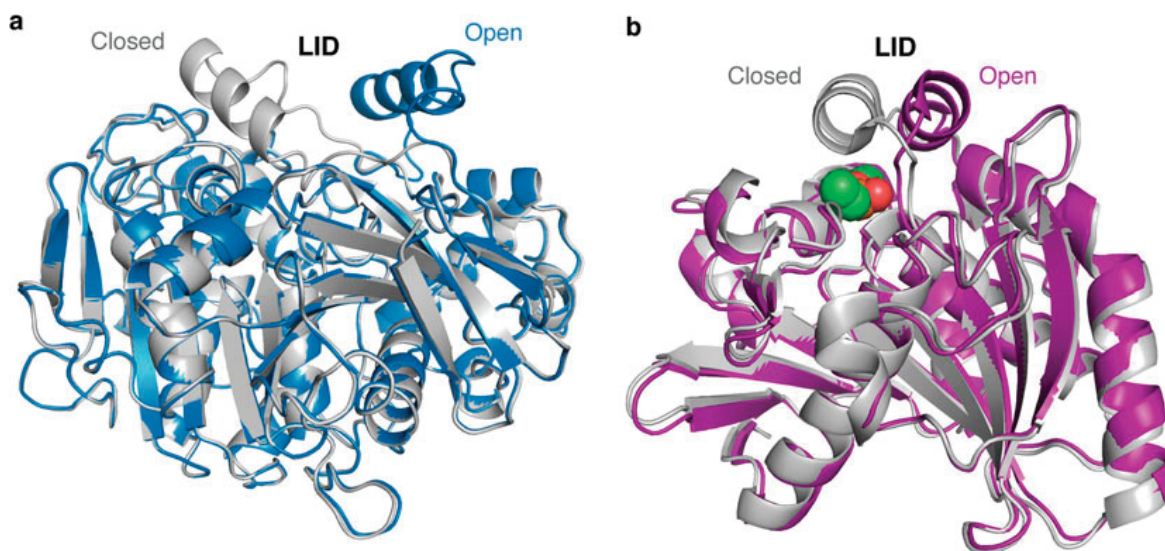


Fig. 4 (a) *C. rugosa* lipase, in blue its open conformation, PDB 1CRL, and in gray its closed conformation, PDB 1TRH; (b) *R. miehei* lipase, in magenta its open conformation with diethyl phosphonate, PDB 4TGL, and in gray its closed conformation, PDB 3TGL

4.4 Lipases α -Helical Loop: The Lid

The resolution of the first three-dimensional structures of lipases from *R. miehei* and human pancreatic lipase [30, 31] enabled the identification of a lid over the active site. The lid is composed of one or more α -helices, joined to the main structure of the enzyme by a flexible structure. It is a mobile element, which uncovers the active site in the presence of a lipid–water interface, generating a conformational change and thus enabling the access of the substrate to the active site [103]. This mechanism, known as interfacial activation, explains the non-Michaelis–Menten behavior observed with most lipases. Indeed, lipase activity increases dramatically when the substrate concentration is high enough to form micelles and emulsions [9] and thus gives sigmoid curves when the reaction initial rate is plotted against the substrate concentration. When the interface is absent, the entrance to the active site is blocked, and the enzyme is inactive. Figure 4 shows the open and closed conformation of the lipases from *C. rugosa* and *R. miehei*. A conformational change is observed in *C. rugosa* lipase in its two conformations (Fig. 4a); for *R. miehei* lipase (Fig. 4b), the lid in its closed conformation obstructs the entrance of the substrate, diethyl phosphonate, while the open lid allows access to the active site. Table 1 gives the amino acids that form the lid of the lipases from *B. cepacia*, *C. rugosa*, *R. miehei*, *R. oryzae*, *Rhizopus delemar*, *T. lanuginosus*, *Y. lipolytica*, and *S. cerevisiae*.

4.5 Substrate-Binding Site

The active site of lipases is located in the inside of a pocket on the top of the central β -sheet of the protein structure [97]. The surface of the pocket's border mainly consists in hydrophobic residues that interact with the hydrophobic substrate. The binding sites of lipases differ in their hydrophobic interaction area, shape, size, deepness of

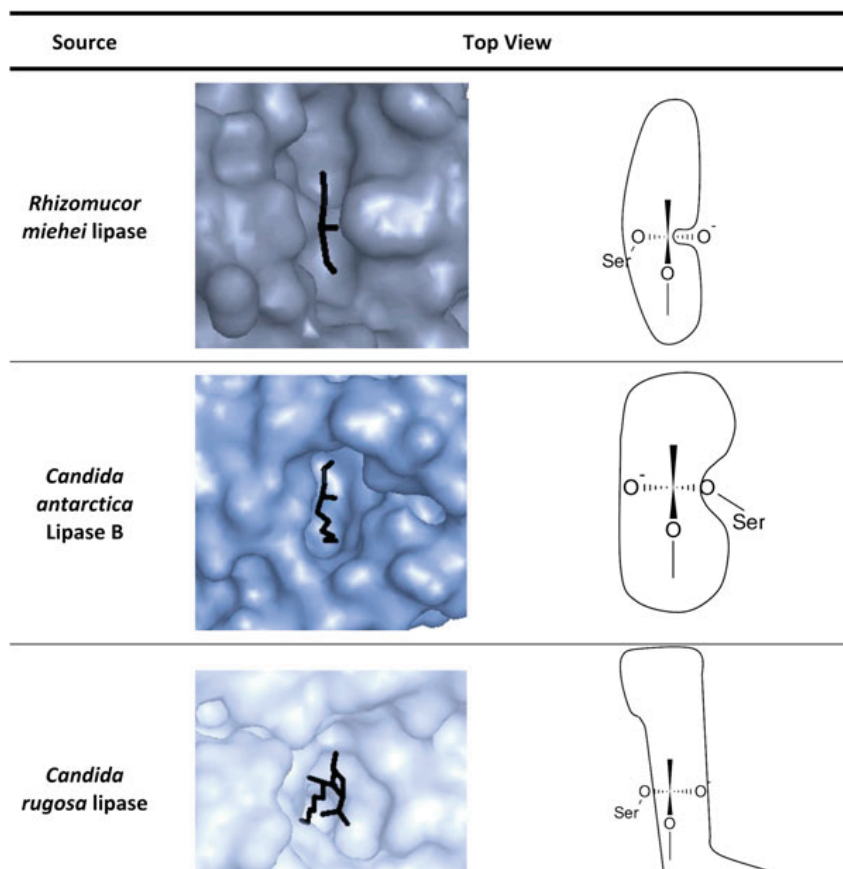


Fig. 5 Shape of the three types of binding site of lipases as identified by [97]

the pocket, and physicochemical characteristics of their amino acids [97]. Pleiss et al. classified lipases into three groups according to the geometry of their binding site (Fig. 5). The first group has a hydrophobic, crevice-like binding site located near the surface of the protein. Lipases from *Rhizomucor* and *Rhizopus* display such a crevice-like binding site. The second group has a funnel-like binding. This group includes lipases from *C. antarctica*, *Burkholderia* sp., and *B. cepacia*, as well as mammalian pancreatic lipases. The last group has a tunnel-like binding site and comprises lipases from *C. rugosa*, for instance.

4.6 Catalytic Mechanism

The catalytic mechanism of lipases is shown in Fig. 6. The mechanism starts by an acylation. This step consists in the transfer of a proton between the aspartate, the histidine, and the serine residues of the lipase, causing the activation of the hydroxyl group of the catalytic serine. As a consequence, the hydroxyl residue of the serine, with subsequently increased nucleophilicity, attacks the carbonyl group of the substrate. The first tetrahedral intermediate is formed with a negative charge on the oxygen of the carbonyl group. The oxyanion hole stabilizes the charge distribution and reduces the state energy of the tetrahedral intermediate by forming

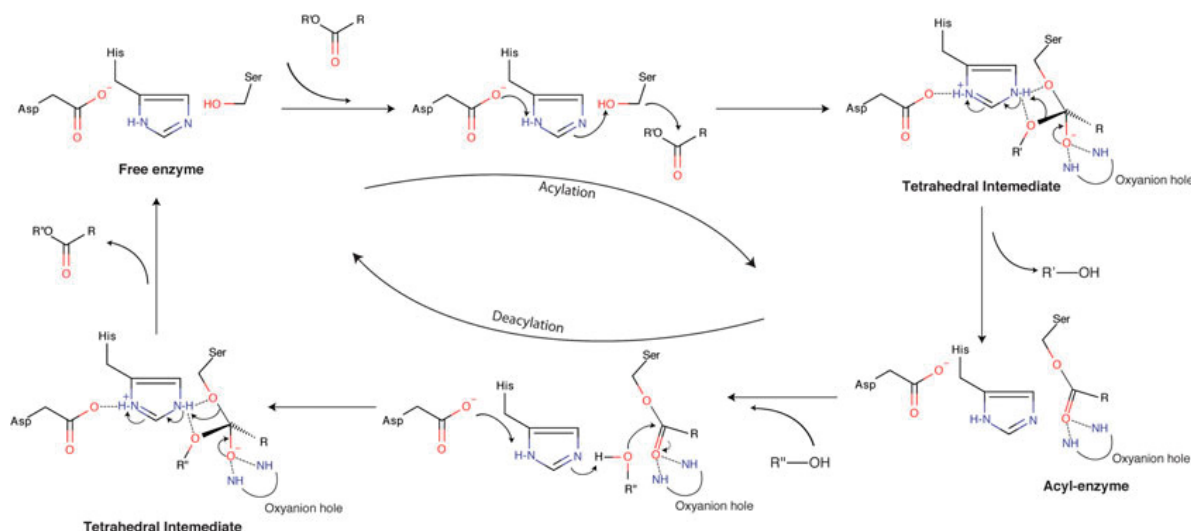


Fig. 6 Catalytic mechanism of lipases

at least two hydrogen bonds. The deacylation step then takes place, where a nucleophile attacks the enzyme, releasing the product and regenerating the enzyme. This nucleophile can be either water in the case of hydrolysis or an alcohol in the case of alcoholysis.

5 Selectivity

Lipase selectivity is related to its preference to perform given reactions. Three types of selectivity can be distinguished: type-selectivity, regioselectivity, and enantioselectivity. The basis of these types of selectivity is discussed below.

5.1 Type-Selectivity

Type-selectivity is associated with the preference for a given substrate, e.g., tri-, di-, or monoglycerides. For instance, *Arabidopsis thaliana* has monoacylglycerol lipases (MAGL), similar to a human MAGL, that hydrolyze the last step of lipid breakdown [112]. Monoacylglycerol lipases have also been found in bacteria since monoglycerides are highly toxic for them [113]. Another lipase with an unusual selectivity is as Lip1 from *Malassezia globosa* which is strictly selective for mono- and diglycerides and is inactive on triacylglycerols [114]. This selectivity also refers to the preference of lipases toward short-, medium-, or long-chain fatty acids and to the degree of unsaturation and potential substitutions of the substrate. The preference of a lipase for acyl groups of different size is directly influenced by the shape of its binding site and the nature of the amino acids composing this binding site. Indeed, the very homologous *C. rugosa* lipase isoforms differ in chain length specificity due to slight modifications of amino acids in their tunnel-shaped binding site [1]. In addition, lipases can show chemo-selectivity which is the specificity of lipases toward a specific chemical group.

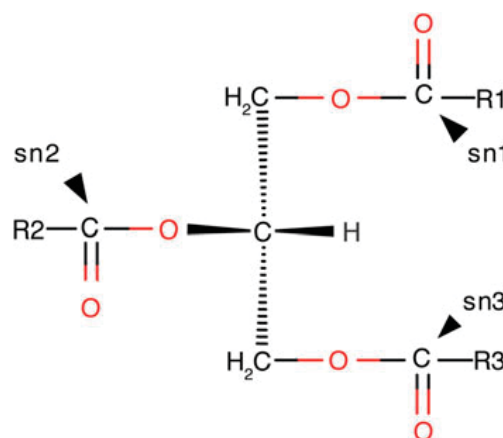


Fig. 7 Identification of the ester bonds potentially hydrolyzed by lipases in a triacylglycerol molecule

5.2 Regioselectivity

Regioselectivity is defined as the preferential attack of lipases toward a given ester bond in the glycerol backbone of triglycerides, i.e., primary or secondary ester bond. Regioselectivity can be *sn*-1 (3) or *sn*-2 (Fig. 7). Lang et al. crystallized the lipase from *B. cepacia* with triglycerides analogues and could unambiguously detect four binding pockets for the triglycerides [73]. The binding pockets include the oxanion hole and three pockets that accommodate the *sn*-1, *sn*-2, and *sn*-3 fatty acid chains. The size and hydrophobicity/hydrophilicity ratio of these different pockets will control the regioselectivity of lipases.

Most microbial lipases hydrolyze the *sn*-1(3) positions of triglycerides, and only few are capable of hydrolyzing the *sn*-2 position. Lipases with *sn*-1(3) specificity include those produced by *Rhizopus oryzae* [115], *Aspergillus niger* [9], *Y. lipolytica* [116], *R. miehei* [117], *Rhizomucor endophyticus* [118], *R. delemar* [119], *T. lanuginosus* [120], and lipase B from *C. antarctica* [121]. Lipases with *sn*-2 specificity are unusual and include those from *Staphylococcus* [122] and lipase C from *Geotrichum* sp. FO401B [123]. Finally, some lipases are nonspecific lipases that act randomly on the triglyceride, producing one molecule of glycerol and three fatty acids. Examples of non-regio-specific lipases are those from *Staphylococcus aureus* [17], *C. rugosa* [1], *C. viscosum*, *P. fluorescens*, *B. glumae*, and *B. cepacia* [9].

5.3 Enantioselectivity

A chiral molecule is a molecule with an asymmetric center, which can adopt two enantiomeric forms, *R* and *S*. Enantiomers *R* and *S* are nonsuperimposable mirror images of each other (Fig. 8), whose chemical properties, such as melting point, solubility, and reactivity, are very similar. However, they often have different biological properties. As a matter of fact, a given enantiomer might show therapeutic activity, while the other might be inactive or even toxic [124]. Enantioselectivity refers to the preference of lipases toward a particular enantiomer of a chiral molecule, in a chemical reaction implying a racemate mixture (mixture of both

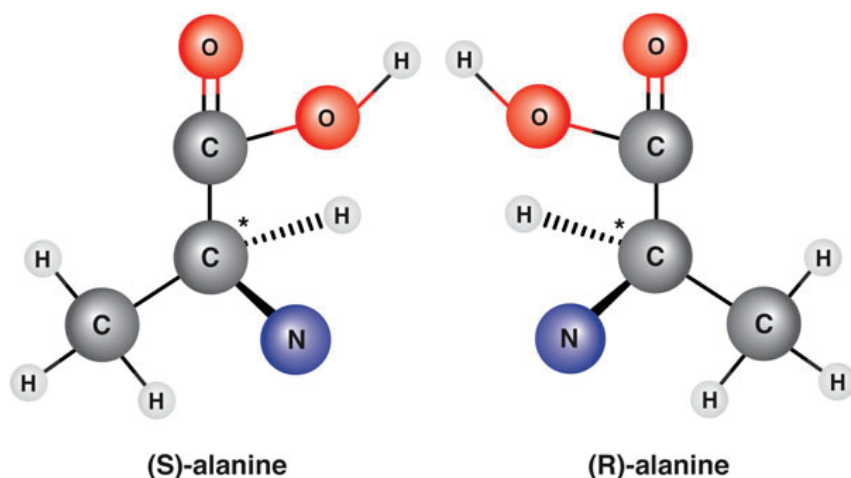


Fig. 8 Representation of a chiral alanine in its two possible enantiomeric forms R and S. The chiral center is represented by an asterisk

enantiomers). Enantioselectivity is thus of great interest in the pharmaceutical industry.

An empirical rule based on the relative size of the substituents at the stereocenter was proposed for the reaction of lipases with secondary alcohols [125]. This rule was also relevant to predict enantioselectivity of lipases for hydrolysis and transesterification reactions, the substrate being an ester or an alcohol, respectively. Unfortunately, rules predicting the enantioselectivity of lipases toward primary alcohols with an oxygen atom attached to the stereocenter are far less reliable [126]. For these types of primary alcohols, a chemoinformatic method gave better predictions than the empirical rule [127]. The X-ray resolution of substrate–enzyme complex structures, additional to molecular modeling of the tetrahedral intermediate or docking of the substrate in the active site, helped deepen the comprehension of lipase enantioselectivity [128]. This strategy was used to predict the enantioselectivity of *B. cepacia* lipase versus secondary alcohols [129]. Recent research showed that the enantioselectivity of lipase B from *C. antarctica*, for the resolution of primary and secondary alcohols, can be controlled by adjusting the polarity of the reaction solvent [130].

By comparing the microbial lipases from *C. antarctica* (lipase B), *R. oryzae*, *R. miehei*, and *C. rugosa* and their interaction with a trioctanoin substrate, Pleiss and collaborators highlighted the importance of both the topology of the binding site (in particular the His-gap motif implying the catalytic histidine) and the structure of the substrate (flexible/rigid character of the *sn*-2 substituent of triglycerols) to explain lipases stereopreference (i.e., *sn*-1 versus *sn*-3 ester bond) [131]. Besides the interaction in the active site and the nature of the substrate, stereospecificity was also shown to be dependent on physicochemical factors such as temperature and solvent. Unfortunately, it is not possible to reasonably explain lipase

enantioselectivity based on their structure or energetic analysis [132, 133]. By the use of an *in silico* approach based on efficient path-planning algorithms, Guieysse et al. explained the enantioselectivity of *B. cepacia* lipase toward (*R,S*)-bromophenylacetic acid ethyl ester by the relative accessibility of the enantiomers to the catalytic center [132]. Recognition of primary alcohols by *B. cepacia* lipase was studied using chemical modification, MALDI-TOF mass spectrometry, and molecular dynamics simulation [134]. Results showed that enantioselectivity of this lipase depends on the hydrogen bond between the oxygen at a non- α -position of the primary alcohol and tyrosine 29. Modification of tyrosine 29 increased the enantioselectivity of *B. cepacia* lipase toward secondary alcohols [135].

6 Applications

Enzymes are key components in a large number of industrial fields. Actually, their worldwide market was estimated in US \$4.9 billions in 2015 [136] and was anticipated to reach US \$7.2 billions in 2020 with an average annual increase of 4.6% per year [137]. The global industrial enzyme market is expected to reach US \$7.0 billions in 2018, with a market distribution of 24.5% in food and beverage, 27.6% in animal feed, 25.5% in detergent, and 22.4% in textile, paper, and pulp [138]. World demand of lipases is expected to reach US \$345 million in 2017, in applications such as baking, biocatalyst, fat and oilseed processing, and diagnostics [139].

Lipases are of great importance in the industry due to their stability in organic solvent, their wide variety of substrates, their selectivity, and their ability to catalyze reactions without addition of expensive cofactors. Moreover, they are also easily produced and active at mild reaction conditions.

Therefore, lipases are used in many different industrial areas such as:

- *Food industry*: Including production of dairy products, such as cheese, modification of fats and oils (e.g., manufacture of butter and margarine, new cooking oils), and production of baby food and structured lipids with unique properties (e.g., cocoa butter equivalent, human milk substitute, high or reduced calorie fats, polyunsaturated fatty acid (PUFA)-enriched oils) [140, 141, 21]. They are used as emulsifiers in the improvement of baked products and pasta and as additives in animal feeding [142]. Finally, they are also used to modify flavors and produce fragrance compounds [21].
- *Detergents and cleaning agents*: As additives since they are active and stable at high temperatures and alkaline pH. They are also essential in the production of soap, dishwashing products, dry cleaning solvents, and contact lens cleaning [16, 143].

- *Fine chemicals*: In the pharmaceutical industry to produce pure enantiomers through resolution of racemic mixtures (e.g., chiral molecules such as prostaglandins, cephalosporins, nonsteroidal anti-inflammatory drugs, hydantoins, and penicillins) [144, 145]. Chiral molecules are also used as herbicides in the agrochemical industry [10]. In the perfume and cosmetic industry, they are employed to produce surfactants and scents and as emollient in personal care products [146].
- *Medical applications*: An alternative application of lipases is as diagnostic tools, since their presence and level can indicate an infection or disease, and as new drugs for treatment of digestive ailments and high cholesterol levels [14].
- *Pulp and paper industry*: In pitch control, for removal of triglycerides and waxes. Moreover, their presence increases whiteness and reduces the pollution in waste waters [14].
- *Lipase bioremediation and environmental processes*: Such as treatment of residual waters rich in oil, degradation of organic debris, and sewage treatment from a wide range of industries [147].
- *Energy industry*: Production of lubricants, biodiesel, and bio-kerosene from renewable sources by transesterification of vegetable/animal oils [148–151]. They are also used to produce additives that decrease the viscosity of biodiesel.
- *Further applications*: Include production of biopolymers [152] such as polyphenols, polysaccharides, and polyesters, lipase-mediated lipophilization, production of biosensors, and modification of phenolic acids and antioxidants. Textile industry also uses lipases for enzymatic wash and jeans treatment [14].

The application of lipases in industrial processes requires, in most cases, an over-expression of the gene of interest in order to obtain larger quantities of the desired lipase. The production method and further purification of the desired enzyme will depend on the quantity and purity needed for a given application. Lipases are mainly produced by submerged fermentation; however they can also be produced by solid-state fermentation [153]. The purification methods used in the industry should be rapid, efficient, inexpensive, and high yielding. Purification methods often involve a first concentration step, also known as pre-purification, by ammonium sulfate precipitation, ultrafiltration, or organic solvent extraction [154]. These techniques generate lipases employed in the cleaning agent industry. However pharmaceutical applications will require highly purified enzymes. Further enzyme purification is mainly achieved by hydrophobic or affinity chromatography, immunopurification, gel filtration, reversed micellar system, and membrane processes [154]. Table 2 summarizes some of the applications and patented processes with lipases. Examples of commercial lipases available in the market are listed in Table 3.

Table 2
Industrial applications of lipases and some patented processes

Industry	Lipases used	Action	Product or application	Patent	References
<i>Food industry³</i>					
Dairy	<i>Aspergillus niger</i> , <i>oryzae</i> , <i>japonicas</i> , and <i>aculeatus</i>	Cheese flavoring and ripening	Flavoring agents (acetoacetate, beta-keto acids, methyl ketones, phenolic aldehydes, flavor esters, and lactones)	WO 2009068098	[155]
	<i>Candida rugosa</i> , <i>utilis</i> , <i>antarctica</i> , and <i>cylindracea</i>	Hydrolysis of milk fat		US 2004033571	[156]
	<i>Penicillium roqueforti</i> and <i>camemberti</i>	Lipolysis and modification of butterfat and cream	Fragrance agents in cheese, milk, and butter	PT 102638	[157]
	<i>Rhizomucor miehei</i> and <i>javanicus</i>			ES 2167205	[158]
	<i>Rhizopus oryzae</i> , <i>niveus</i> , and <i>chinensis</i>			ES 2149689	[159]
	<i>Pseudomonas</i> sp.			US 6162623	[160]
	<i>Pseudomona cepacia</i> and <i>fluorescens</i>			US 3973042	[161]
	Porcine pancreas			WO 2009106575	[162]
	<i>Thermomyces lanuginosa</i>			CN 104531781	[163]
	<i>Chromobacterium viscosum</i>			CN 104480148	[164]
	<i>Aspergillus oryzae</i>			US 20160319259	[165]
	<i>Fusarium oxysporum</i>				
Fats and oils		Transesterification	Butter substitutes (cocoa butter)	EP 0191217	[166]
		Hydrolysis	Glycerides for butter and margarine	KR 20090031740	[167]
		Esterification	SL rich in PUFA	CA 1318624	[168]
			Low caloric triglycerols	US 2006141592	[169]
			Chocolate tempering improvement	JP 2004283043	[170]
			Milk fat substitutes	CN 102634547	[171]
			Concentrate or purified FA	CN 105219813	[172]
			Diglycerols for cooking oils	WO 03040091	[173]
			Formation of monoacylglycerol and diacylglycerol	US 2002197687	[174]
			Omega 3-PUFA-enriched oil	CA 1050908	[175]
			Etolide production	WO 2012087153	[176]
				WO 2014013335	[177]
				WO 2016151115	

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Table 2
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Industry	Lipases used	Action	Product or application	Patent	References
Baked products and confectionery		Flavor improvement	Extend shelf-life	US 2003180418	[178]
		Control	Increase loaf volume	CA 1050908	[174]
		non-enzymatic	Improve crumb structure	WO 2007096201	[179]
		browning	Mayonnaise and dressings	RO 121070	[180]
		Quality	Emulsifiers	EP 1586240	[181]
		improvement		WO 2013122599	[182]
				WO 2013135822	[183]
				WO 2014161876	[184]
Others		Transesterification	Sausage manufacture, ripening	WO 2013167573	[185]
		Hydrolysis	Improve aroma, flavor, and fermentation	WO 2014124509	[186]
		Synthesis	Alcoholic beverages (e.g., apple wine, beer)	CN 105230754	[187]
Detergents			Sugar esters		
			Improving flour		
	<i>Acinetobacter</i> sp. <i>Aspergillus oryzae</i> <i>Candida</i> sp. <i>Chromobacterium</i> sp. <i>Pseudomonas mendocina</i> , <i>pseudocaligenes, alcaligenes, cepacia</i> , and <i>wisconsinensis</i> <i>Thermomyces lanuginosus</i> <i>Humicola insolens</i> <i>Streptomyces rimosus, coelicolor</i> , <i>and griseus</i> <i>Geobacillus stearothermophilus</i> <i>Thermobifida fusca</i> <i>Psychromonas ingrahamii</i> Genetically engineered variants	Hydrolysis	Fats removal (decomposition of lipids)	US 6017866	[188]
			Soap production	WO 9708281	[189]
			Dishwashing, dry cleaning solvents, liquid	WO 9600292	[190]
			leather cleaner, contact lens cleaning	US 5763383	[191]
			Enzymatic bleaching system	EP 0385401	[192]
				EP 2787066	[193]
				US 20140193886	[194]
				CN 104024393	[195]
				CN 105209612	[196]
				US 20170073619	[197]
				WO 2014087011	[198]
				WO 2013171241	[199]
				WO 2013116261	[200]
				WO 2011156297	[201]
				WO 2011150157	[202]
				WO 2011084417	[203]

Table 2
(continued)

Industry	Lipases used	Action	Product or application	Patent	References
Cosmetics and perfumes	<i>Rhizomucor miehei</i>	Synthesis of additive Esterification	Emollient in creams and bath oils	DE 102007039736	[237]
	<i>Candida rugosa</i> and <i>antarctica</i> B		Anti-obese creams	WO2012087153	[175]
	<i>Pseudomonas cepacia</i>		Emulsifiers	CN103114108	[238]
	<i>Rhizopus oryzae</i>		Moisturizing	WO2015065354	[239]
			Surfactants	CN102876740	[240]
<i>Pulp and paper industry</i>					
	<i>Candida rugosa</i>	Hydrolysis	Pitch control	US 2010269989	[241]
	<i>Pseudomonas</i> sp.		Wastepaper deinking	WO 2007035481	[242]
			Increase paper whiteness	WO 2006029404	[243]
			Reduce waste water pollution	US 2003124710	[244]
			Control deposition of organic contaminants on fiber	WO 2013191803	[245]
<i>Bioremediation</i>					
Waste treatment	<i>Acinetobacter calcoaceticus</i>	Hydrolysis	Remove fat layer	DE 19834359	[246]
	<i>Candida rugosa</i>		Degreasing (e.g., holding tanks)	EP 1707540	[247]
	<i>Pseudomonas cepacia</i> and <i>aeruginosa</i>		Degradation of organic debris	DE 10261349	[248]
	<i>Yarrowia lipolytica</i>		Clearing of drains	FR 2846984	[249]
	<i>Rhizopus oryzae</i>		Sewage treatment plants	EP 2593407	[250]
Oil biodegradation	<i>Acinetobacter</i> sp.	Biodegradation of petroleum hydrocarbons	Water reconditioning		
	<i>Mycobacterium</i> sp.		Beta-lactam antibiotic inactivation		
	<i>Rhodococcus</i> sp.		Degradation of oil spills (e.g., <i>n</i> -alkanes, aromatic hydrocarbons, and polycyclic aromatic hydrocarbons)		[251]
<i>Energy</i>					
	<i>Aspergillus niger</i> and <i>tubingensis</i>	Transesterification	Biodiesel	CN 1687313	[252]
	<i>Candida rugosa</i> , <i>antarctica</i> , and	Esterification	Reduction of biodiesel viscosity	CN 101381614	[253]

<i>deformans</i> <i>Thermomyces lanuginosa</i> <i>Rhizomucor javanicus</i> and <i>miebei</i> <i>Penicillium camemberti</i> and <i>cepacia</i> <i>Rhizopus oryzae</i> and <i>niveus</i> <i>Staphylococcus haemolyticus</i> <i>Streptomyces</i> sp. Genetically engineered variants	Lubricants	CN 101260417	[254]	
	Alcohol esters	CN 101250424	[255]	
		CN 101240201	[256]	
		JP 2006272326	[257]	
		WO 2012173658	[258]	
		WO 2012106701	[259]	
		WO 2012098114	[260]	
		EP 2657324	[261]	
		KR 20130136066	[262]	
		US 2013260428	[263]	
		KR 20130055477	[264]	
		CN 105296446	[265]	
<i>Farming</i>				
Agriculture	<i>Bacillus firmus</i>	Plant parasite control agent	WO 2014043058	[266]
Animal feed	<i>Aspergillus tubingensis</i> and <i>niger</i>	Feed supplement	US 8735123	[222]
		Aquaculture supplement	US 2013280228	[267]
			CN 105614168	[268]
<i>Others</i>				
Polymers	<i>Candida antarctica</i> Porcine pancreas	Biodegradable polyesters	US 2010048927	[269]
		Aromatic polyesters	US 20140273111	[270]
		Lubricants	US 20140196631	[271]
		Functionalized polymers	WO 2014099438	[272]
Textiles	Removal of lubricants Stone and enzymatic washing Bio polishing	Jeans, yarns, fabrics, rugs		[273]
Medical application	<i>Aspergillus niger</i> <i>Candida rugosa</i> and <i>antarctica</i> <i>Galleria mellonella</i> <i>Serratia marcescens</i>	Diagnostic tools	WO 2004018660	[274]
		Treatment of gastrointestinal disturbances	US 5075231	[275]
		Treatment malignant tumors	US 2010216212	[276]
		Cholesterol lowering drug	WO 2008079685	[234]
				(continued)

(continued)

Table 2
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Industry	Lipases used	Action	Product or application	Patent	References
	<i>Porcine pancreas</i>	laboratory samples	Treatment wounds and microbial infection	CN 101518646	[277]
	<i>Candida cylindracea</i>	Nitric oxide	Treatment pancreatic enzyme insufficiency	CN 104459164	[278]
	Recombinant human lysosomal	precursor formation	Treatment lysosomal acid lipase deficiency	US 20110104240	[279]
				US 20080279839	[280]
				CA 2849250	[281]
				US 20120064055	[282]
Leather degreasing	<i>Rhizopus nodosus</i>	Hydrolysis of fats	Waste water treatment		[283]
	<i>Candida rugosa</i>		Water proof leather		

^aThe lipases listed are used in all food industry applications

Table 3
Commercially available lipases

Type	Source	Form	Producing company
Fungal	<i>Candida rugosa</i>	Powder	Atlas Biologics, Amano, Biocatalysts, Boehringer Mannheim, Meito Sangyo, Fluka, Genzyme, Sigma, Advanced Enzyme Technologies Ltd.
	<i>Candida antarctica</i> B	Powder	Advanced Enzyme Technologies Ltd.
	<i>Candida antarctica</i> A/B	Immobilized	Boehringer Mannheim, Novo Nordisk, Sigma
	<i>Thermomyces lanuginosus</i>	Immobilized	Novo Nordisk, Boehringer Mannheim
	<i>Rhizomucor miehei</i>	Immobilized/liquid	Novo Nordisk, Biocatalysts, Amano, Chr. Hansen
	<i>Yarrowia lipolytica</i>	Powder	Amano, Artechno S.A.
	<i>Geotrichum candidum</i>	Liquid	Boehringer Mannheim, Novo Nordisk
	<i>Rhizopus</i> sp.	Powder	Yiming Biological Products Co. Ltd.
	<i>Aspergillus oryzae</i>	Powder	Suntaq International Ltd.
Bacterial	<i>Aspergillus niger</i>	Powder	Sunson Industry Group Company Limited
	<i>Burkholderia cepacia</i>	Powder	Amano, Fluka, Boehringer Mannheim
	<i>Pseudomonas alcaligenes</i>	Powder	Gist-Brocades, Genencor International
	<i>Pseudomonas mendocina</i>	Powder	Genencor International
Animal	<i>Chromobacterium viscosum</i>	Liquid	Asahi, Biocatalysts
	Pig pancreatic lipase	Granulated	Solvay pharma, Biocatalyst
	Calf, kid goat, and lamb	Powder	Chr. Hansen, Clerici-Sacco Group, Danlac Canada Inc.
Unknown source/ non-stated by company			Winovazyme Biological Science & Technology Co., Ltd., SternEnzym, DuPont-Danisco, Leveking Enzymes, Ran Industrias Químicas S.A., Fenchem, Inc., Pakmaya, Royal DSM NV, Bukman Laboratories Inc., Aum Enzymes

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