NRC Research Press

MINIREVIEW

Classification of lipolytic enzymes and their biotechnological applications in the pulping industry

L. Ramnath, B. Sithole, and R. Govinden

Abstract: In the pulp and paper industry, during the manufacturing process, the agglomeration of pitch particles (composed of triglycerides, fatty acids, and esters) leads to the formation of black pitch deposits in the pulp and on machinery, which impacts on the process and pulp quality. Traditional methods of pitch prevention and treatment are no longer feasible due to environmental impact and cost. Consequently, there is a need for more efficient and environmentally friendly approaches. The application of lipolytic enzymes, such as lipases and esterases, could be the sustainable solution to this problem. Therefore, an understanding of their structure, mechanism, and sources are essential. In this report, we review the microbial sources for the different groups of lipolytic enzymes, the differences between lipases and esterases, and their potential applications in the pulping industry.

Key words: lipases, esterases, lipolytic, microbial, biopulping.

Résumé : Dans l'industrie des pâtes et papiers, durant le processus de fabrication, l'agglomération de particules de poix (composées de triglycérides, d'acides gras et d'esters) conduit à la formation de dépôts de poix noire dans la pâte et dans la machinerie, ce qui a des retombées sur la chaîne de production et sur la qualité de la pâte à papier. Les méthodes traditionnelles de prévention et de traitement de la poix ne sont plus envisageables en raison de leur impact environnemental et de leur coût. On aurait donc besoin d'approches plus efficientes et respectueuses de l'environnement. Une solution durable à ce problème pourrait résider dans l'application d'enzymes lipolytiques comme des lipases et des estérases. Dès lors, il est essentiel de bien comprendre leur structure, leur fonctionnement et leurs sources. La présente revue traite des sources microbiennes de divers groupes d'enzymes lipolytiques, des différences entre les lipases et les estérases, et de leurs applications potentielles dans l'industrie papetière. [Traduit par la Rédaction]

Mots-clés : lipases, estérases, lipolytiques, microbien, pulpation biologique.

Introduction

Enzymes are a vital component of numerous industrial processes, such as pulp and paper, food, beverage, detergent, clothing, fuel, and pharmaceutical manufacturing (Gurung et al. 2013). Currently, hydrolases are the most popular enzymes in biotechnology (Gurung et al. 2013), with carbohydrases, proteases, and lipases forming more than 70% of all enzyme sales in the current market, which is expected to reach US$10.7 billion by 2024 (Global Market Insights 2016; Li et al. 2012). Hydrolases are a class of enzymes that demonstrate broad substrate specificity. They are capable of hydrolyzing peptides, halides, amides, as well as esters and triglycerides (Fojan et al. 2000). One of the most valuable classes of hydrolases in biotechnological applications are lipolytic enzymes, which comprise lipases (EC 3.1.1.1, triacylglycerol hydrolases) and "true" esterases (EC 3.1.1.3, carboxyl ester hydrolases) (Casas-Godoy et al. 2012; Fan et al. 2012; Hudlicky and Reed 2009; Bornscheuer 2002). Lipases and esterases are known to catalyze both the hydrolysis and synthesis of ester compounds. They are characteristically resilient enzymes that can withstand harsh industrial processes with wide pH ranges, high temperatures, and the presence of organic solvents (Gupta et al. 2004). They also exhibit characteristic chemo-, stereo-, and regioselectivity that are of particular interest in applications, such as the synthesis of optically pure compounds (Haki and Rakshit 2003). Their catalytic flexibility, robustness, and high specificity attract a lot of attention as industrial biocatalysts. This report is a review of the different types of lipolytic enzymes, their mechanisms of action, and

Received 12 July 2016. Revision received 25 October 2016. Accepted 25 October 2016.

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their potential application in various industrial processes, with an emphasis on the pulp and paper industry.

Lipolytic families

The 3-dimensional structure of lipases and esterases displays the characteristic α/β-hydrolase fold (Ollis et al. 1992) and a definite order of α-helices and β-sheets. Bacterial lipolytic enzymes are classified into 8 families (families I–VIII) based on variances in their amino acid sequences and biological properties (Arpigny and Jaeger 1999). Of the 8 different lipolytic families, family I ("true" lipases — interfacial activation and presence of a lid is characteristic of this family) is the largest and is further divided into 6 subfamilies. Carboxyl esterases along with various other lipases are grouped into the other 7 families, e.g., family II (also called Gly-Asp-Ser-(Leu) [GDSL] (sequence motif) family), family III, family IV (also called HSL (hormone-sensitive lipase) family), family V, family VI, family VII, and family VIII (Arpigny and Jaeger 1999).

Lipolytic family I

Lipolytic family I is the most represented family and is divided into 7 subfamilies, with an estimated total of 27 members. Lipases of this family share a Gly-Xaa-Ser-Xaa-Gly (GXSXG) consensus sequence (Kanaya et al. 1998; Østerlund et al. 1996). Family I includes the "true" lipases, e.g., Gram-negative *Pseudomonas* lipases and lipases from Gram-positive bacteria, such as *Bacillus*, *Staphylococcus*, *Propionibacterium*, and *Streptomyces* (Arpigny and Jaeger 1999). The expression of active lipases belonging to subfamilies I.1 and I.2 is dependent on a chaperone protein called lipase-specific foldase (Lif). Two aspartic residues are implicated in the Ca-binding site found at homologous positions in all sequences, and the 2 cysteine residues forming a disulfide bridge are conserved in most sequences. These 4 residues are believed to be important in stabilizing the active site of these enzymes (Kim et al. 1997). An example of such a lipase (LipC12) (Ogierman et al. 1997) belongs to family I.1 of bacterial lipases, which has a chaperone-independent folding, does not possess disulfide bridges, and is calcium ion dependent. LipC12 is stable from pH 6 to 11 and has activity from pH 4.5 to 10, with higher activities at alkaline pH values. Stability is observed in environments with a NaCl concentration of up to 3.7 mol/L and at temperatures ranging from 20 to 50 °C, with maximum activity at 30 °C over a 1 h incubation period. The pure enzyme has specific activities of 1767 and 1722 U/mg against pig fat and olive oil, respectively (Glogauer et al. 2011).

Bacillus lipases are generally classified under 2 subfamilies: I.4 and I.5 (Arpigny and Jaeger 1999). Several *Bacillus* lipases are known to have a common alanine residue that replaces the first glycine in the conserved pentapeptide: Ala-Xaa-Ser-Xaa-Gly. However, *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus licheniformis* lipases from the I.4 subfamily are the smallest true lipases (approx. 19–20 kDa) and they share very little similarity at the amino acid level (approx. 15%) with the other *Bacillus* and *Staphylococcus* lipases (Arpigny and Jaeger 1999). Temperatures above 45 °C result in decreased activities for subfamily I.4 lipases; however, they display high activity at basic pH (between 9.5 and 12) (Guncheva and Zhiryakova 2011). Some exceptions include the lipases of *Bacillus thermocatenulatus*, which have a molecular mass of about 43 kDa and display maximal activity at approx. 65 °C and pH 9.0 (Carrasco-Lopez et al. 2009; Schmidt-Dannert et al. 1994). Another *Bacillus* sp., known as *Bacillus stearothermophilus*, also produces a lipase with a molecular mass of 67 kDa and optimal activity at pH 11 and 55 °C (Bacha et al. 2015). Massadeh and Sabra (2011) reported maximal lipase activity of 90.57 U/mL from *B. stearothermophilus* at 45 °C and pH 8. Other examples of lipases in this family are produced by staphylococcal isolates. They produce lipases larger than that produced by *Bacillus* sp. (approx. 75 kDa) that are secreted as precursors and cleaved in the extracellular medium by a specific protease, generating a protein of approximately 400 residues. The pro-peptide (207 ± 267 residues) seemingly operates as an intramolecular chaperone and assists in translocation of the lipase across the cell membrane (Sørensen and Mortensen 2005; Götz et al. 1998).

Lipolytic family II

Lipolytic family II has 5 members that have been characterized and reported. Enzymes categorized as family II do not demonstrate the usual pentapeptide (GXSXG) but exhibit a GDS(L) motif consisting of the active-site serine residue. This residue lies much closer to the N terminus in these proteins than in other lipolytic enzymes (Upton and Buckley 1995). Unlike conventional lipases, GDSL enzymes do not possess a nucleophile elbow, which is a region containing a β–β–α structural motif and holds the nucleophilic and the oxyanion hole amino acid residues that form the catalytic site in various enzymes. The nucleophile (Ser, Asp, or Cys) is found in a sharp turn, the so-called nucleophile elbow. GDSL enzymes are known to have flexible active sites that are able to modify its structure in the presence of specific substrates, thus increasing its substrate specific range (Akoh et al. 2004). Included in this family are esterases of *Streptomyces scabies*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Photorhabdus luminescens*, *Vibrio mimicus*, *Escherichia coli*, and *Aeromonas hydrophila* (Henderson et al. 1998; Loveless and Saier 1997) and lipases of *A. hydrophilia*, *Vibrio parahemolyticus*, *Xenorhabdus luminescens*, and *Streptomyces rimosus* (Akoh et al. 2004). An example of an extracellular lipase from this family was isolated from *P. aeruginosa*, with a molecular mass of 30 kDa. The lipase was stable up to 45 °C and maintained activity in the alkaline pH range (Sharon et al. 1998). The enzyme was found to be highly stable in the presence of methanol and ethanol, and cationic surfactants, such as Triton X-100 and Tween 80, substantially increased activity. The presence of a calcium-binding site in this *Pseudomonas* lipase is predicted, as its activity was stabilized significantly by Ca^{2+} and inactivation of the

enzyme by EDTA was overcome by subsequent $CaCl_2$ treatment (Sharon et al. 1998).

Lipolytic family III

Family III of lipolytic enzymes comprises 3 characterized members so far. Enzymes possess the conserved consensus sequence GXSXG. Members of this family are very closely related (Lee 2016). Enzymes exhibit the canonical α/β-hydrolase fold and contain a typical catalytic triad. High activities at low temperature (less than 15 °C) were believed to originate from conserved sequence motifs of these enzymes (Langin et al. 1993; Feller et al. 1991). However, distinct sequence similarity between esterases from psychrophilic (*Moraxella* sp., *Psychrobacter immobilis*), mesophilic (*Alcaligenes eutrophus*, *E. coli*), and thermophilic (*Alicyclobacillus acidocaldarius*, *Archeoglobus fulgidus*) microorganisms suggest that temperature variation is not responsible for such considerable sequence conservation (Arpigny and Jaeger 1999). A comparative study of these enzymes would be beneficial in resolving the unique properties of this family of hydrolases. An example of an enzyme from this family is the extracellular lipase from *Serratia marcescens*, ECU1010, with a molecular mass of 65 kDa, a pI of 4.2, and pH and temperature optima of 8.0 and 45 °C, respectively (Zhao et al. 2008).

Lipolytic family IV

Family IV, otherwise known as the hormone-sensitive lipase (HSL) lipolytic family, due to its high sequence similarity to the mammalian HSL (Hausmann and Jaeger 2010), has 6 characterized members. The hydrolysis reaction of triacylglycerols in adipose tissue is catalyzed by HSL, in addition to being a rate-limiting enzyme in the exclusion of fatty acids from stored lipids (Østerlund et al. 1996). This family has 2 highly conserved consensus motifs, the common GXSXG and His-Gly-Gly [HGG], which play a role in the oxyanion hole formation (Mohamed et al. 2013). Enzymes are known to exhibit an α/β-hydrolase fold (Ngo et al. 2013). Enzymes from this family have been identified in *Pseudomonas* sp., *A. eutrophus*, *Moraxella* sp., *A. acidocaldarius*, *E. coli*, and *A. fulgidus* (Manco et al. 2000, 2001; Choo et al. 1998; Kanaya et al. 1998; Feller et al. 1991). An esterase of *Pyrobaculum calidifontis* is considered as a member of the HSL family. Activities reported for this enzyme are similar if not higher than previous reports of 1050 U/mg at 30 °C and 6410 U/mg at 90 °C (Hotta et al. 2002; Rashid et al. 2001; Manco et al. 2000).

Lipolytic family V

Enzymes in this family are made up of 6 characterized members to date. Some of them originate from mesophilic (*Acetobacter pasteurianus*, *Pseudomonas oleororans*, *Haemophilus influenza*), psychrophilic (*Sulfolobus acidocaldarius*), and thermophilic microorganisms (Arpigny and Jaeger 1999). Typically, this family possesses the conserved motif GXSXG, as well as the other common motif PTL (Nacke et al. 2011). A lipolytic enzyme (EstV) from *Helicobacter pylori* has been isolated, cloned, purified, and classified as a family V hydrolase. This enzyme was predominantly active with short-chain substrates (*p*-nitrophenol acetate, *p*-nitrophenol butyrate, *p*-nitrophenol valerate) and did not display interfacial activation, but was stable and had a maximum activity at 50 °C and pH 10 (Ruiz et al. 2007).

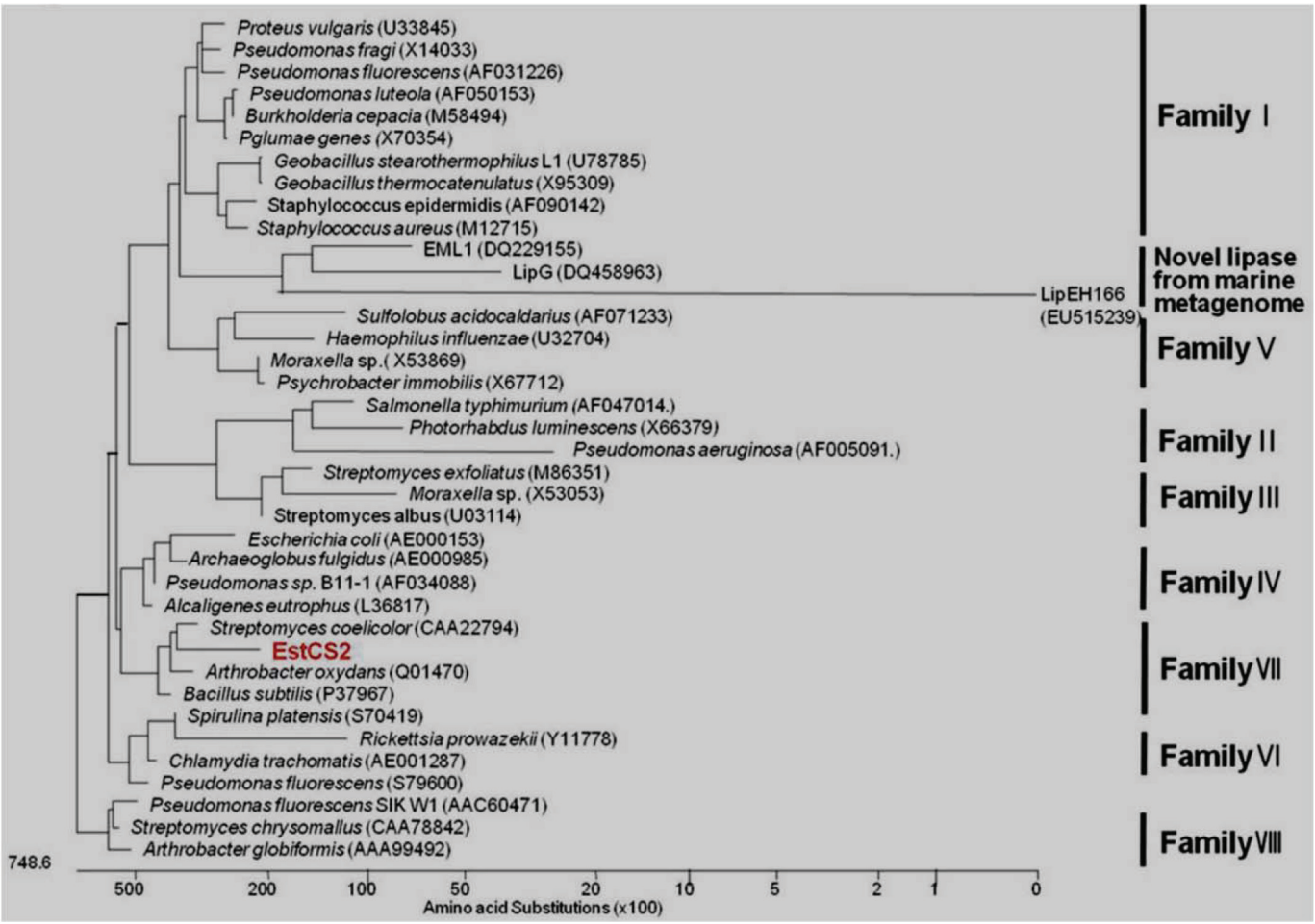
Lipolytic family VI

There are 5 well-characterized members that are classified as family VI lipolytic enzymes based on their size. This family consists of the smallest esterases, with a molecular mass of 23 ± 26 kDa. The subunit has the α/β-hydrolase fold and a classical Ser-Asp-His catalytic triad. A carboxylesterase from *P. fluorescens* is an example of such an esterase (Kim et al. 1997). This enzyme hydrolyzes small ester-containing compounds with a broad specificity and demonstrates no activity towards long-chain triglycerides (Hong et al. 1991). In another example, an extracellular alkaline lipase produced by *P. fluorescens* AK102 was stable between pH 4 and 10 with an optimum pH between 8 and 10 and an optimum temperature of 55 °C. This enzyme could have potential application in the pulping industry for the removal of pitch (Kojima et al. 1994). There is a lack of information on other enzymes in this family. Interestingly, esterases from this family show 40% homology at the amino acid level to eukaryotic lysophospholipases (Bornscheuer 2002), which are responsible for the liberation of fatty acids from lysophospholipids (Schmiel and Miller 1999).

Lipolytic family VII

Family VII esterases are a smaller family with only 3 well-known members. They have an estimated size of ±55 kDa and share substantial amino acid sequence homology (30% identity, 40% similarity) with eukaryotic acetylcholine esterases and intestine and liver carboxylesterases (Arpigny and Jaeger 1999). This family has the classical triad GXSXG pentapeptide. The esterase from *Arthrobacter oxydans* is classified under this family and is specifically active against phenylcarbamate herbicides by hydrolyzing the central carbamate bond (Pohlenz et al. 1992). The esterase is plasmid-encoded and thus has the potential to be more easily transferred to other strains or species. Interestingly, the esterase of *B. subtilis* is capable of hydrolyzing *p*-nitrobenzyl esters, and it may be applied in the final removal of *p*-nitrobenzyl ester utilized as a protecting group in the synthesis of β-lactam antibiotics (Zock et al. 1994). Another esterase isolated from a *Bacillus* strain was found to be thermostable and had maximum activity at 60 °C and maintained 100% activity at 75 °C for 30 min (Andualema and Gessesse 2012). These characteristics would be appropriate for application in the reduction of pitch during the pulping process (high process temperatures) where glyceride lipids may be targeted.

Fig. 1. Phylogenetic tree based on a novel amino acid sequence of EstCS2 and closely related proteins. Protein sequences for previously identified families of bacterial lipolytic enzymes retrieved from GenBank (http://www.ncbi.nlm.nih.gov). The units at the bottom of the tree indicate the number of substitution events. Reproduced with permission from figure 1b in Kang et al. (2011).



Lipolytic family VIII

Family VIII consists of 3 characterized members. These enzymes are approximately 380 aa in length with similarity to many class C β-lactamases. A 150 aa fragment (from positions 50 to 200) showed 45% similarity at the amino acid level to an *Enterobacter cloacae ampC* gene product (Galleni et al. 1988). The *ampC* gene codes for resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations (Jacoby 2009). This trait indicates that the active site (Ser-Xaa-Xaa-Lys) conserved in the N-terminal, belongs to class C β-lactamases (Nishizawa et al. 1995; Lobkovsky et al. 1993). Contrary to this, the esterase or lipase consensus sequence (GXSXG) of the *P. fluorescens* esterase was proposed to be involved in the active site of β-lactamases (Kim et al. 1994). Esterases from *Streptococcus chrysomallus* also possess this motif, but it is not conserved in the *Arthrobacter globiformis* esterase. Also, the motif is situated in close proximity to the C-terminus of the *P. fluorescens* and *S. chrysomallus* enzymes, with an absence of the histidine attachment (amino acid used in the synthesis of proteins). This demonstrates the unconventional nature of these enzymes, as the Ser-Asp-His residue sequence is conserved throughout the entire superfamily of lipases and esterases. Site-directed mutagenesis studies have demonstrated that the (GXSXG) motif does not play a significant role in enzyme functioning of an esterase (EstB) from *Burkholderia gladioli* (Petersen et al. 2001).

A number of genera, such as *Pseudomonas* and *Streptomyces*, are known to produce hydrolases, which are classified into different families (Fig. 1). Arpigny and Jaeger (1999) first classified bacterial lipolytic enzymes into the 8 families according to their amino acid sequences and biological properties. This became the reference point for classification of novel lipolytic enzymes to a family. However, unique families are being discovered through the use of metagenomics (Fu et al. 2011; Kim et al. 2009; Lee et al. 2006). Table 1 summarizes the different classes of lipolytic enzymes currently described.

Lipases

Lipases (e.g., triacylglycerol hydrolase, EC 3.1.1.3) hydrolyze long-chain acyl groups (7C10) to fatty acids and

Table 1. Current classification of bacterial lipolytic enzymes.

Family	Description
I	Group of true lipases subdivided into 6 subfamilies: *Pseudomonas* lipases and relatives (subfamilies I.1, I.2, and I.3), *Bacillus* and *Staphylococcus* lipases and relatives (I.4 and I.5) and other lipases (I.6).
II	Modified pentapeptide motif around the active serine: Gly-Asp-Ser-(Leu) [GDS(L)]; secreted and membrane-bound esterases.
III	Extracellular lipases and esterases.
EstA*	Related to family III but different conserved motifs (pentapeptide Gly-His-Ser-Met-Gly) [GHSMG]; discovered from surface seawater.
IV	Many members of this family show sequence similarity to mammalian hormone-sensitive lipase (HSL); typical motif His-Gly-Gly [HGG]; lipolytic enzymes from psychrophilic, mesophilic, and thermophilic origins.
EstB*	New subfamily in family IV with second active site glutamate (conserved sequence Glu-X-Leu-Leu-Asp [EXLLD]) instead of the aspartate Asp-Pro-Leu-X-Asp (DPLXD) of the representative members of family IV; it was discovered in surface sea water.
V	Conserved motif His-Gly-Gly-Gly [HGGG] upstream of the pentapeptide motif Gly-Asp-Ser-Ala-Gly [GDSAG]; sequence similarity with nonlipolytic enzymes: epoxide hydrolases, dehalogenases, and haloperoxidases; esterases from psychrophilic, mesophilic, and thermophilic origins.
EstF*	Related to family V but with a modified pentapeptide, Gly-Thr-Ser-X-Gly [GTSXG], and different flanking regions around the HG motif and their own unique conserved sequence motifs; isolated from deep sea sediments.
VI	The smallest esterases known (23–26 kDa); sequence similarity to eukaryotic lysophospholipases.
VII	Large bacterial esterases (55 kDa); sequence homology with eukaryotic acetylcholine esterases and intestine and (or) liver carboxylesterases.
VIII	Similarity to several class C β-lactamases.
LipG*	Presence of an Arg-Gly sequence in oxyanion hole instead of His-Gly, a signature sequence distinctive of filamentous fungal lipases; isolated from tidal flat sediments.
LipEH166*	Comprise newly discovered lipase LipEH166 of psychrophilic origin, and three putative open reading frames; isolated from intertidal flat sediments.
EstY*	Derived from pathogenic bacteria; first possible lipolytic virulence factors that do not belong to the GDSL family; isolated from surface river water.

Note: Description of bacterial lipolytic families I–VIII in the Arpigny and Jaeger classification, and new families and subfamily discovered by functional metagenomics (indicated by an asterisk (*)) (adapted from Fu et al. 2011; Lee et al. 2006; Kim et al. 2009; Arpigny and Jaeger 1999).

acylglycerols (Ellaiah et al. 2004; Bornscheuer 2002; Litthauer et al. 2002; Verger 1997; Ollis et al. 1992). Lipases can be differentiated from esterases by the occurrence of interfacial activation, which is only observed in lipases, whereas esterases obey classical Michaelis–Menten kinetics. Structural analysis revealed that this interfacial activation is due to a hydrophobic domain (lid) covering the active site of lipases; however, only in the presence of a minimum substrate concentration, i.e., only in the presence of triglycerides or hydrophobic organic solvents, does the lid move apart, making the active site available (Sharma et al. 2001; Jaeger et al. 1999). Therefore, lipases have altered properties from esterases, which have an acyl binding pocket (Pleiss et al. 1998).

Microbial lipases specifically have unlimited potential in commercial applications, such as additives in fine chemicals, wastewater treatment, food processing, cosmetics, detergents, pharmaceuticals, degreasing formulations, paper manufacture, and accelerated degradation of fatty wastes and polyurethane (Rodriques et al. 2016; Shu et al. 2016; Speranza et al. 2016; Fulton et al. 2015; Adulkar and Rathod 2014; Gerits et al. 2014; Li et al. 2014; Saranya et al. 2014; Brabcova et al. 2013; Lailaja and Chandrasekaran 2013; Nerurkar et al. 2013; Whangsuk et al. 2013; Zhang et al. 2013; Liu et al. 2012; Kamini et al. 2000). Even though a large number of lipases have been described in the literature, only for a limited number of enzymes belonging to a few species has it been proven that their stability and biosynthetic activity is amenable for use in organic solvents, and thus for consideration as industrially applicable enzymes (Cardenas et al. 2001; Jaeger et al. 1999). Their biotechnological potential is dependent on their capacity to catalyze not only the hydrolysis of triglycerides but also their synthesis from glycerol and fatty acids (Jaeger et al. 1999). Lipases are known to hydrolyze up to 90% of triglycerides in pitch to glycerol or monoglycerides and fatty acids, which are considerably less sticky and more hydrophilic (simple to wash) than the triglycerides (Jaeger and Reetz 1998).

Classification

Classification of lipolytic enzymes as "true" lipases requires the fulfilment of 2 criteria. (*i*) They should be activated by the presence of an interface, i.e., their activities should dramatically increase as soon as the triglyceride substrate forms an emulsion. This occurrence is termed "interfacial activation". (*ii*) They should also contain a "lid", which is a surface loop of the protein covering the active site of the enzyme and moves away on contact with the interface (van Tilbeurgh et al. 1993; Derewenda et al. 1992; Brzozowski et al. 1991). The "true" lipase family (family I) covers the 6 subfamilies that predominantly catalyze the hydrolytic reactions of substrates with long acyl chains (Messaoudi et al. 2011).

Mechanism of action of lipases

Lipases take effect on ester bonds present in acylglycerols to release free fatty acids and glycerol in a liquid medium (Villeneuve et al. 2000). In limited liquid environments, these enzymes are capable of reversing this reaction (esterification) via acidolysis, interesterification, and alcoholysis (Villeneuve et al. 2000). Elucidation of their structures revealed that the interfacial activation observed is due to a hydrophobic domain (lid) covering the active site of lipases and that high levels of activity were observed only in the presence of a minimum substrate concentration (Araújo et al. 2008).

Microorganisms producing lipases

Lipases have originated from plants, animals, and microorganisms; however, bacterial lipases are the most versatile, stable, and reactive in organic medium (Andualema and Gessesse 2012). Numerous microorganisms are known to produce lipases when incubated with lipid substrates (Haba et al. 2000). The majority of bacterial lipases originate from Gram-negative bacteria, the most valuable being *Pseudomonas*, which includes at least 7 lipase-producing species, which are *P. aeruginosa* (Prasad 2014; Tielen et al. 2013), *Pseudomonas cepacia* (Badgujar et al. 2016; Cao et al. 2016; Sasso et al. 2016), *Pseudomonas alcaligenes* (Chen et al. 2014; Patel et al. 2014), *Pseudomonas glumae* (Knapp et al. 2016), *P. fluorescens* (Guldhe et al. 2015; Lima et al. 2015; Xun et al. 2012), *Pseudomonas fragi* (Dey et al. 2014; Santarossa et al. 2005), and *Pseudomonas putida* (Fatima et al. 2014).

Heterologous production of lipases

Lipases from *Pseudomonas* species require the functional assistance of about 30 different cellular proteins prior to recovery from the culture supernatant in an enzymatically active state, demonstrating that folding and secretion are highly specific processes that generally do not function properly in heterologous hosts (Rosenau et al. 2004; Rosenau and Jaeger 2000). Extracellular enzymes, such as lipases, must be translocated through the bacterial membrane to a suitable location to fulfil their function. Gram-positive bacteria secrete enzymes which cross a single cytoplasmic membrane. These proteins generally contain a signal sequence, directing translocation via the secretion machinery (Fekkes and Driessen 1999). The TAT pathway is a second translocation pathway found in lipase-secreting Gram-negative and Gram-positive bacteria (Shruthi et al. 2010; Heravi et al. 2009). Proteins utilizing this pathway contain a unique twin arginine translocation motif in their signal sequence (Tjalsma et al. 2000). In general, active expression of lipases from *Pseudomonas* and *Burkholderia* requires the presence of a chaperone protein known as the lipase-specific foldase (Lif), for precise folding of the lipase (Quyen et al. 2012; Wu et al. 2012). A text book example would be that of the cold-active lipase gene isolated from *Psychrobacter* sp., which was expressed in *E. coli* BL21 yielding a specific activity of 66.51 U/mg. When the recombinant plasmid was co-expressed with a "chaperone team", the lipase displayed a specific activity of 108.77 U/mg (Cui et al. 2011). Other expression hosts, such as *Bacillus* species, have also been explored. A lipase isolated from *P. vulgaris* was expressed in *B. subtilis* WB800, which displayed a high lipase activity of 356.8 U/mL after 72 h induction with sucrose (Lu et al. 2010). Lipolytic genes have also been isolated from metagenomes and expressed in different host strains (Liaw et al. 2010). A lipase from a metagenome has been cloned in *Streptomyces lividans* with maximal activity of 4287 U/mg towards *p*-nitrophenyl butyrate at 60 °C and pH 8.5 (Cote and Shareck 2010).

Esterases

Esterases retain significant applications in various biotechnological processes due to their stability in organic solvents, extensive substrate specificity, stereoselectivity, regioselectivity, and lack of requirement for cofactors (Akoh et al. 2004; Jaeger and Eggert 2002; Faber 2000; Patel 2000). Esterases (EC 3.1.1.1) hydrolyze the ester bonds of water-soluble fatty acid esters with short-chain acyl groups (6C8) (Verger 1997; Ollis et al. 1992). Several methods have been developed to screen and isolate novel esterases (Kumar et al. 2012; Elend et al. 2006; Kim et al. 2006; Choi et al. 2003; Suzuki et al. 2003), including metagenomic techniques (Henne et al. 2000). A range of esterase characteristics have been described, primarily in molecular biology, targeted synthesis, purification, quantitation, production, and distribution.

Mechanism of action of esterases

Esterases boast a diversity of substrate specificities; however, they typically possess a catalytic triad composed of Ser, His, and Asp or Glu in the polypeptide chain (Bornscheuer 2002; Verger 1997; Ollis et al. 1992). The active site Ser residue is integrated at the centre of the conserved pentapeptide sequence motif, GXSXG (Ollis et al. 1992). The motif is usually positioned in the sharp turn between a β-strand and α-helix, known as the nucleophilic elbow (Verger 1997). Ester bond hydrolysis is mediated by the nucleophilic attack of the active Ser on the carbonyl of the substrate in a charge-relay system with the 2 other amino acid residues (His and Asp or Glu) (Ollis et al. 1992). Ester hydrolysis or formation is fundamentally the same for lipases and esterases and involves 4 steps. Initially, the substrate is attached to the active serine, generating a tetrahedral intermediate that is stabilized by the catalytic His and Asp residues. Next, the alcohol is liberated and an acyl-enzyme complex is produced. Attack of a nucleophile forms a tetrahedral intermediate again, which following resolution, generates the product (an acid or an ester) and free enzyme (Bornscheuer and Kazlauskas 1999). In some cases, esterases may appear to function in vitro as esterases; however, they may end up functioning in vivo as transferases. An ideal example is that of the *O*-acetyl

peptidoglycan esterase 2 (Ape2) protein in *Neisseria gonorrhoeae*, which was thought to be an *O*-acetyl peptidoglycan esterase but instead functioned as a peptidoglycan *O*-acetyltransferase (Moynihan and Clarke 2010). This may prove to be a major obstacle in the implementation of enzymes for biotechnological applications.

Microorganisms producing esterases

Esterases are produced by an array of organisms, such as *Streptomyces* sp. (Nishimura and Inouye 2000), *Pseudomonas* sp. (Prim et al. 2006; Tserovska et al. 2006), *Bacillus* sp. (Ding et al. 2014; Metin et al. 2006), *Lactobacillus* sp. (Choi and Lee 2001), *Thermoanaerobacterium* sp. (Shao and Wiegel 1995), *Micrococcus* sp. (Fernández et al. 2004), *Ophistoma* sp. (Calero-Rueda et al. 2002), *Penicillium* sp. (Horne et al. 2002), *Aspergillus* sp. (Giuliani et al. 2001), *Humicola* sp. (Hatzakis et al. 2003), *Sporotrichum* sp. (Topakas et al. 2003), *Saccharomyces* sp. (Lomolino et al. 2003), *Candida* sp. (Ghosh et al. 1991), plants (Pringle and Dickstein 2004), and animals (Finer et al. 2004), and may be applied in valuable biological processes. The efficient hydrolysis of both triglycerides and sterol esters using sterol esterase from *Ophiostoma piceae* has been successfully applied for pitch control in the pulp and paper industry (Coloma et al. 2015; Gutiérrez-Fernández et al. 2014; Calero-Rueda et al. 2002). Steryl and cholesteryl esterases from *Pseudomonas* sp. (Sugihara et al. 2002; Svendsen et al. 1995; Uwajima and Terada 1976), *Chromobacterium viscosum* (Kontkanen et al. 2004), and *Candida rugosa* (Rúa et al. 1993) are also reported to contribute a vital role in reducing pitch problems during paper manufacturing.

Heterologous production of esterases

Numerous reports have been published on the cloning and expression of microbial esterases (Brod et al. 2010; Huang et al. 2010; Terahara et al. 2010; Ro et al. 2004; Khalameyzer et al. 1999). When considering heterologous expression, one needs to investigate the type of expression system to be applied, as this could have a significant effect on the level of enzymes produced. For instance, *O. piceae* produces a sterol esterase with high activity in the hydrolysis of triglycerides and sterol esters; however, once expressed in *Pichia pastoris*, greater activity is observed due to higher solubility (Cedillo et al. 2012). This is due to the alteration in the N-terminal sequence of the protein expressed in *P. pastoris*, which includes 4–8 additional amino acids, which ultimately modified its aggregation performance (Vaquero et al. 2015).

In 2004, Choi and colleagues discovered a novel esterase gene (*estI*). The amino acid sequence indicated that it may be classified as a novel member of the GHSMG family of lipolytic enzymes. *Escherichia coli* BL21 (DE3)/pLysS containing the *estI* gene expressed a novel 67.5 kDa protein corresponding to EstI in an N-terminal fusion with the S-tag peptide. The optimum pH and temperature of the purified enzyme were 7.0 and 37 °C, respectively. The most specific substrate was *p*-nitrophenyl-caprylate (C8) with K_m and k_{cat} values of approximately 14 μmol/L and 1245 s^{-1}, respectively (Choi et al. 2004).

Moukouli et al. (2008) successfully cloned a Type C feruloyl esterase in *Saccharomyces cerevisiae* under the transcriptional control of the alcohol oxidase (AOX1) promoter and integrated it into *P. pastoris* X-33 to validate feruloyl esterase activity. A recombinant protein with a molecular mass of 62 kDa and a pI of 6.8 was produced (Moukouli et al. 2008). When incubated together with the xylanase from *Trichoderma longibrachiatum* in de-starched wheat bran, ferulic acid was effectively liberated. The esterase displayed broad pH stability making it a significant applicant for alkaline applications such as pulp treatment in the paper industry (Moukouli et al. 2008).

The *axe* gene, which encodes an acetylxylan esterase from *Thermobifida fusca* NTU22, has been cloned, sequenced, and expressed in *E. coli*. The optimal pH and temperature of the purified esterase was 7.5 and 60 °C, respectively. A significant increase in xylooligosaccharide production was observed when oat–spelt xylan was treated with a combination of the recombinant xylanase and acetylxylan esterase compared with independent treatment with xylanase or acetylxylan esterase (Huang et al. 2010). A sterol esterase from *O. piceae* was expressed in 2 hosts: *S. cerevisiae* and *P. pastoris*. The highest activity of 42 U/mL was produced by *P. pastoris* at 28 °C; however, low activity was observed in *S. cerevisiae*. The heterologous expression of a functional fungal esterase in yeast is quite an accomplishment and opens up an opportunity to develop more robust enzymes (Vaquero et al. 2015).

Applications

Enzymes are currently being applied in multiple industries. The flexibility of enzyme properties enables application in a number of degradation and synthesizing processes, such as the detergent, food, animal feed, beverage, textile, pulp and paper, organic synthesis, and leather industries (Table 2). An international initiative towards industrial biotechnology has opened up a niche for the development of novel enzymes. Enzymes are designed based on the limiting parameters of processes and on their stability and effectiveness in catalyzing specific reactions.

Lipases in particular have enormous potential in the lipid bio-industry because of their availability and stability in organic media (Sharma and Kanwar 2014). The application of lipases in the pulp and paper making industry is vital in the control of pitch formation. Pitch is described as the agglomeration of lipophilic extractives from wood material and causes black spots in the pulp and gumming up of machinery. Esterases on the other hand are used to break down the polyvinyl acetate in glues present in recycled paper processing (Jegannathan and Nielsen 2013). The use of such enzymes in the pulp and paper industry reduces the consumption of energy and

Table 2. Enzymes used in various industrial processes and their application.

Industry	Enzyme	Application
Detergent	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipase	Lipid stain removal
	Cellulase	Cleaning, colour clarification
	Mannanase	Reappearing stains
Starch and fuel	Amylase	Starch liquefaction and saccharification
	Amyloglucosidase	Saccharification
	Pullulanase	Saccharification
	Glucose isomerase	Glucose to fructose conversion
	Cyclodextrin-glycosyltransferase	Cyclodextrin production
	Xylanase	Viscosity reduction
	Protease	Protease (yeast nutrition – fuel)
	Lipase	Synthesis of lipase-catalyzed biodiesel
Food	Protease	Milk clotting, flavour
	Lipase	Improvement of food texture
	Lactase	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Baking	Amylase	Bread softness and volume
	Xylanase	Dough conditioning
	Lipase	Dough stability and conditioning
	Phospholipase	Dough stability and conditioning
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Dough strengthening, bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths
Animal feed	Phytase	Phytate digestibility
	Xylanase	Digestibility
	β-Glucanase	Digestibility
Beverage	Pectinase	Depectinization, mashing
	Amylase	Juice treatment, low calorie beer
	β-Glucanase	Mashing
	Acetolactate decarboxylase	Maturation (beer)
	Laccase	Clarification (juice), flavour (beer)
Textile	Cellulase	Denim finishing, cotton softening
	Amylase	Desizing
	Pectate lyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal
Pulp and paper	Lipase	Pitch control, contaminant control
	Protease	Biofilm removal
	Amylase	Starch coating, deinking, drainage improvement
	Xylanase	Bleach boosting
	Cellulase	Deinking, drainage improvement, fiber modification
Fats and oils	Lipase	Transesterification
	Phospholipase	Degumming, lysolecithin production
Organic synthesis	Lipase	Resolution of chiral alcohols and amides
	Acylase	Synthesis of semisynthetic penicillin
	Nitrilase	Synthesis of enantiopure carboxylic acids
Leather	Protease	Unhearing, bating
	Lipase	Depickling
Personal care	Amyloglucosidase	Antimicrobial
	Glucose oxidase	Bleaching, antimicrobial
	Peroxidase	Antimicrobial
Environmental application	Lipase	Degradation of lipid wastes Removal of solid and water pollution by hydrocarbons, oils, and lipids

Note: adapted from Gurung et al. 2013; Nigam 2013; Sarrouh et al. 2012; Imran et al. 2012; Vijayalakshmi et al. 2011; Ramteke et al. 2005; Chang et al. 2004; Cavicchioloi and Siddiqui 2004; Kirk et al. 2002; Margesin et al. 2002.

amount of chemicals used in processing, thus limiting their negative contribution to the environment (Skals et al. 2008; Patrick 2004). In the recycling of newspaper, the paper needs to be deinked prior to production of newsprint and white paper (Bajpai 2013). When the ink is composed of vegetable oil, lipases are often used to break down the lipophilic components of the ink (Hasan et al. 2006; Yang et al. 1994). Yang et al. (1994) patented a specific combination of parameters and ratio of cellulases, xylanases, and lipases for the removal of ink from various paper samples while preserving the quality of the salvaged pulp.

Recent advances in metagenomics and proteomics has supported the discovery of novel enzymes and genetic engineering of microbes (Adrio and Demain 2014). Future applications of enzymes from microbial, plant, and animal resources will ensure a more feasible approach to bioprocessing, as well as reducing the amount of waste generated and overall impact on the environment.

Acknowledgements

This work was funded by the National Research Foundation. The authors declare no conflict of interest.

References

Adrio, J.L., and Demain, A.L. 2014. Microbial enzymes: tools for biotechnological processes. Biomolecules, **4**: 117–139. doi:10.3390/biom4010117. PMID:24970208.

Adulkar, T.V., and Rathod, V.K. 2014. Ultrasound assisted enzymatic pre-treatment of high fat content dairy wastewater. Ultrason. Sonochem. **21**: 1083–1089. doi:10.1016/j.ultsonch.2013.11.017. PMID:24380806.

Akoh, C.C., Lee, G.-C., Liaw, Y.-C., Huang, T.-H., and Shaw, J.-F. 2004. GDSL family of serine esterases/lipases. Prog. Lipid Res. **43**: 534–552. doi:10.1016/j.plipres.2004.09.002. PMID:15522763.

Andualema, B., and Gessesse, A. 2012. Microbial lipases and their industrial applications: review. Biotechnology, **11**: 100–118. doi:10.3923/biotech.2012.100.118.

Araújo, R., Casal, M., and Cavaco-Paulo, A. 2008. Application of enzymes for textiles fibres processing. Biocatal. Biotechnol. **26**: 332–349. doi:10.1080/10242420802390457.

Arpigny, J.L., and Jaeger, K.E. 1999. Bacterial lipolytic enzymes: classification and properties. Biochem. J. **343**: 177–183. doi:10.1042/bj3430177. PMID:10493927.

Bacha, B.A., Moubayed, N.M., and Abid, I. 2015. Thermostable, alkaline and detergent-tolerant lipase from a newly isolated thermophilic *Bacillus stearothermophilus*. Indian J. Biochem. Biophys. **52**: 179–88. PMID:26118130.

Black, E.L. 2000. Resin in suspensions and mechanisms of its deposition. Chap. 6. *In* Pitch control, wood resin and deresination. *Edited by* E.L. Black and L.H. Allen. Tappi Press, Atlanta, Ga., USA. pp. 170–174.

Badgujar, K.C., Pai, P.A., and Bhanage, B.M. 2016. Enhanced biocatalytic activity of immobilized *Pseudomonas cepacia* lipase under sonicated condition. Bioprocess Biosyst. Eng. **39**: 211–221. doi:10.1007/s00449-015-1505-5. PMID:26590966.

Bajpai, P. 2013. Recycling and deinking of recovered paper. Elsevier Science, Amsterdam, the Netherlands.

Bornscheuer, U.T. 2002. Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiol. Rev. **26**: 73–81. doi:10.1111/j.1574-6976.2002.tb00599.x. PMID:12007643.

Bornscheuer, U.T., and Kazlauskas, R.J. 1999. Hydrolases in organic synthesis — regio- and stereoselective biotransformations. Wiley-VCH, Berlin, Germany.

Brabcova, J., Filice, M., Gutarra, M., and Palomo, J.M. 2013. Synthesis of enantiopure drugs and drug intermediates by immobilized lipase-catalysis. Curr. Bioact. Compd. **9**: 113–136. doi:10.2174/22115528112019990009.

Brod, F.C.A., Vernal, J., Bertoldo, J.B., Terenzi, H., and Arisi, A.C.M. 2010. Cloning, expression, purification, and characterization of a novel esterase from *Lactobacillus plantarum*. Mol. Biotechnol. **44**: 242–249. doi:10.1007/s12033-009-9232-2. PMID:20033357.

Brzozowski, A.M., Derewenda, U., Derewenda, Z.S., Dodson, G.G., Lawson, D.M., Turkenburg, J.P., et al. 1991. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. Nature, **351**: 491–494. doi:10.1038/351491a0. PMID:2046751.

Calero-Rueda, O., Plou, F.J., Ballesteros, A., Martinez, A.T., and Martinez, M.J. 2002. Production, isolation and characterization of a sterol esterase from *Ophiostoma piceae*. Biochem. Biophys. Acta, **1599**: 28–35. PMID:12479402.

Cao, S.-L., Huang, Y.-M., Li, X.-H., Xu, P., Wu, H., Li, N., et al. 2016. Preparation and characterization of immobilized lipase from *Pseudomonas cepacia* onto magnetic cellulose nanocrystals. Sci. Rep. **6**: 20420. doi:10.1038/srep20420. PMID:26843037.

Cardenas, J., Alvarez, E., de Castro-Alvarez, M.-S., Sanchez-Montero, J.-M., Valmaseda, M., Elson, S.W., and Sinisterra, J.-V. 2001. Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. J. Mol. Catal. B: Enzym. **14**: 111–123. doi:10.1016/S1381-1177(00)00244-7.

Carrasco-Lopez, C., Godoy, C., de Las Rivas, B., Fernandez-Lorente, G., Palomo, J.M., Guisan, J.M., et al. 2009. Activation of bacterial thermoalkalophilic lipases is spurred by dramatic structural rearrangements. J. Biol. Chem. **284**: 4365–4372. doi:10.1074/jbc.M808268200. PMID:19056729.

Casas-Godoy, L., Duquesne, S., Bordes, F., Sandoval, G., and Marty, A. 2012. Lipases: an overview. Methods Mol. Biol. **861**: 3–30. doi:10.1007/978-1-61779-600-5_1. PMID:22426709.

Cavicchioli, R., and Siddiqui, K.S. 2004. Cold adapted enzymes. *In* Enzyme technology. *Edited by* A. Pandey, C. Webb, C.R. Soccol, and C. Larroche. Asiatech Publishers, India. pp. 615–638.

Cedillo, V.B., Plou, F.J., and Martínez, M.J. 2012. Recombinant sterol esterase from *Ophiostoma piceae*: an improved biocatalyst expressed in *Pichia pastoris*. Microb. Cell Fact. **11**: 73–87. doi:10.1186/1475-2859-11-73. PMID:22676486.

Chang, H., Liao, H., Lee, C., and Shieh, C. 2004. Optimized synthesis of lipasecatalyzed biodiesel by Novozym 435. J. Chem. Technol. Biotechnol. **80**: 307–312. doi:10.1002/jctb.1166.

Chen, H., Wu, J., Yang, L., and Xu, G. 2014. Characterization and structure basis of *Pseudomonas alcaligenes* lipase's enantiopreference towards D,L-methyl propionate. J. Mol. Catal. B: Enzym. **102**: 81–87. doi:10.1016/j.molcatb.2014.01.020.

Choi, G.-S., Kim, J.-Y., Kim, J.-H., Ryu, Y.-W., and Kim, G.-J. 2003. Construction and characterization of a recombinant esterase with high activity and enantioselectivity to (S)-ketoprofen ethyl ester. Protein Expression Purif. **29**: 85–93. doi:10.1016/S1046-5928(03)00009-3.

Choi, Y.-J., and Lee, B.H. 2001. Culture conditions for the production of esterase from *Lactobacillus casei* CL96. Bioprocess Biosyst. Eng. **24**: 59–63. doi:10.1007/s004490100233.

Choi, Y.J., Míguez, C.B., and Lee, B.H. 2004. Characterization and heterologous gene expression of a novel esterase from *Lactobacillus casei* CL96. Appl. Environ. Microbiol. **70**: 3213–3221. doi:10.1128/AEM.70.6.3213-3221.2004. PMID:15184114.

Choo, D.W., Kurihara, T., Suzuki, T., Soda, K., and Esaki, N. 1998. A cold-adapted lipase of an Alaskan psychrotroph, *Pseudomonas* sp. strain B11-1: gene cloning and enzyme purification and char-

acterization. Appl. Environ. Microbiol. **64**: 486–491. PMID: 9464382.

Coloma, J., Reyes, L., Navarrete, J., Alarcón, J., Delgado, L., Vera, R., et al. 2015. Effect of albino *Ophiostoma* strains on *Eucalyptus nitens* extractives. Maderas: Cienc. Tecnol. **17**: 161–170. doi:10.4067/S0718-221X2015005000016.

Cote, A., and Shareck, F. 2010. Expression and characterization of a novel heterologous moderately thermostable lipase derived from metagenomics in *Streptomyces lividans*. J. Ind. Microbiol. Biotechnol. **37**: 883–891. doi:10.1007/s10295-010-0735-4. PMID:20495942.

Cui, S.-s., Lin, X.-z., and Shen, J.-h. 2011. Effects of co-expression of molecular chaperones on heterologous soluble expression of the cold-active lipase Lip-948. Protein Expression Purif. **77**: 166–172. doi:10.1016/j.pep.2011.01.009.

Derewenda, U., Brzozowski, A.M., Lawson, D.M., and Derewenda, Z.S. 1992. Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase. Biochemistry, **31**: 1532–1541. doi:10.1021/bi00120a034. PMID:1737010.

Dey, A., Chattopadhyay, A., Mukhopadhyay, S.K., Saha, P., Chatterjee, S., Maiti, T.K., and Roy, P. 2014. Production, partial purification and characterization of an extracellular psychrotrophic lipase from *Pseudomonas* sp. ADT3. J. Biorem. Biodegrad. **5**: 242. doi:10.4172/2155-6199.1000242.

Ding, J., Yu, T., Liang, L., Xie, Z., Yang, Y., Zhou, J., et al. 2014. Biochemical characterization of a GDSL-motif esterase from *Bacillus* sp. K91 with a new putative catalytic mechanism. J. Microbiol. Biotechnol. **24**: 1551–1558. PMID:25394512.

Elend, C., Schmeisser, C., Leggewie, C., Babiak, P., Carballeira, J.D., Steele, H.L., et al. 2006. Isolation and biochemical characterization of two novel metagenome-derived esterases. Appl. Environ. Microbiol. **72**: 3637–3645. doi:10.1128/AEM.72.5.3637-3645.2006. PMID:16672512.

Ellaiah, P., Prabhakar, T., Ramakrishna, B., Taleb, A.T., and Adinarayana, K. 2004. Production of lipase by immobilized cells of *Aspergillus niger*. Process Biochem. **39**: 525–528. doi:10.1016/S0032-9592(01)00340-5.

Faber, K. 2000. Biotransformations in organic chemistry. 4th ed. Springer, Berlin, Germany. doi:10.1007/978-3-642-98071-8.

Fan, X., Niehus, X., and Sandoval, G. 2012. Lipases as biocatalyst for biodiesel production. Methods Mol. Biol. **861**: 471–483. doi:10.1007/978-1-61779-600-5_27. PMID:22426735.

Fatima, H., Khan, N., Rehman, A.U., and Hussain, Z. 2014. Production and partial characterization of lipase from *Pseudomonas putida*. Ferment. Technol. **2**: 112. doi:10.4172/2167-7972.1000112.

Fekkes, P., and Driessen, A.J. 1999. Protein targeting to the bacterial cytoplasmic membrane. Microbiol. Mol. Biol. Rev. **63**: 161–173. PMID:10066835.

Feller, G., Thiry, M., Arpigny, J.L., and Gerday, C. 1991. Cloning and expression in *Escherichia coli* of three lipase-encoding genes from the psychrotrophic Antarctic strain *Moraxella* TA144. Gene, **102**: 111–115. doi:10.1016/0378-1119(91)90548-P. PMID:1864500.

Fernández, J., Mohedano, A.F., Fernández-García, E., Medina, M., and Nuñez, M. 2004. Purification and characterization of an extracellular tributyrin esterase produced by a cheese isolate, *Micrococcus* sp. INIA 528. Int. Dairy J. **14**: 135–142. doi:10.1016/S0958-6946(03)00168-7.

Finer, Y., Jaffer, F., and Santerre, J.P. 2004. Mutual influence of cholesterol esterase and pseudocholinesterase on the biodegradation of dental composites. Biomaterials, **25**: 1787–1793. doi:10.1016/j.biomaterials.2003.08.029. PMID:14738842.

Fojan, P., Jonson, P.H., Petersen, M.T.N., and Petersen, S.B. 2000. What distinguishes an esterase from a lipase: a novel structural approach. Biochemie, **82**: 1033–1041. doi:10.1016/S0300-9084(00)01188-3.

Fu, C., Hu, Y., Xie, F., Guo, H., Ashforth, E.J., Polyak, S.W., et al. 2011. Molecular cloning and characterization of a new cold-active esterase from a deep-sea metagenomic library. Appl. Microbiol. Biotechnol. **90**: 961–970. doi:10.1007/s00253-010-3079-0. PMID:21336688.

Fulton, A., Frauenkron-Machedjou, V.J., Skoczinski, P., Wilhelm, S., Zhu, L., Schwaneberg, U., and Jaeger, K.E. 2015. Exploring the protein stability landscape: *Bacillus subtilis* lipase A as a model for detergent tolerance. ChemBioChem, **16**: 930–936. doi:10.1002/cbic.201402664. PMID:25773356.

Galleni, M., Lindberg, F., Normark, S., Cole, S., Honore, N., Joris, B., and Frere, J.-M. 1988. Sequence and comparative analysis of three *Enterobacter cloacae ampC* β-lactamase genes and their products. Biochem. J. **250**: 753–760. doi:10.1042/bj2500753. PMID:3260487.

Gerits, L.R., Pareyt, B., Decamps, K., and Delcour, J.A. 2014. Lipases and their functionality in the production of wheat-based food systems. Compr. Rev. Food Sci. Food Saf. **13**: 978–989. doi:10.1111/1541-4337.12085.

Ghosh, D., Erman, M., and Duax, W.L. 1991. Crystallization and preliminary diffraction analysis of cholesterol esterase from *Candida cylindracea*. J. Steroid Biochem. Mol. Biol. **38**: 663–665. doi:10.1016/0960-0760(91)90325-Y. PMID:2039758.

Giuliani, S., Piana, C., Setti, L., Hochkoeppler, A., Pifferi, P.G., Williamson, G., and Faulds, C.B. 2001. Synthesis of pentylferulate by a feruloyl esterase from *Aspergillus niger* using water-in-oil microemulsions. Biotechnol. Lett. **23**: 325–330. doi:10.1023/A:1005629127480.

Global Market Insights. 2016. Enzymes Market Size By Product (Proteases, Lipases, Carbohydrases, Polymerases & Nucleases), By Application (Detergents, Food & beverages, Animal Feed, Biofuels), Industry Analysis Report, Regional Outlook (U.S., Canada, Germany, UK, France, Italy, Sweden, Austria, Poland, Russia, China, India, Japan, Indonesia, Thailand, Malaysia, Australia, Brazil, Argentina, Mexico, South Africa, UAE, Saudi Arabia, Kuwait, Egypt), Growth Potential, Price Trends, Competitive Market Share & Forecast, 2016–2024. Report ID: GMI743. Available from https://www.gminsights.com/industry-analysis/enzymes-market.

Glogauer, A., Martini, V.P., Faoro, H., Couto, G.H., Müller-Santos, M., Monteiro, R.A., et al. 2011. Identification and characterization of a new true lipase isolated through metagenomic approach. Microb. Cell Fact. **10**: 54. doi:10.1186/1475-2859-10-54. PMID:21762508.

Götz, F., Verheij, H.M., and Rosenstein, R. 1998. Staphylococcal lipases: molecular characterization, secretion, and processing. Chem. Phys. Lipids, **93**: 15–25. doi:10.1016/S0009-3084(98)00025-5. PMID:9720246.

Guldhe, A., Singh, B., Rawat, I., Permaul, K., and Bux, F. 2015. Biocatalytic conversion of lipids from microalgae *Scenedesmus obliquus* to biodiesel using *Pseudomonas fluorescens* lipase. Fuel, **147**: 117–124. doi:10.1016/j.fuel.2015.01.049.

Guncheva, M., and Zhiryakova, D. 2011. Catalytic properties and potential applications of *Bacillus lipases*. J. Mol. Catal. B: Enzym. **68**: 1–21. doi:10.1016/j.molcatb.2010.09.002.

Gupta, R., Gupta, N., and Rathi, P. 2004. Bacterial lipases: an overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. **64**: 763–781. PMID:14966663.

Gurung, N., Ray, S., Bose, S., and Rai, V. 2013. A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Res. Int. doi:10.1155/2013/329121.

Gutiérrez-Fernández, J., Vaquero, M.E., Prieto, A., Barriuso, J., Martínez, M.J., and Hermoso, J.A. 2014. Crystal structures of *Ophiostoma piceae* sterol esterase: structural insights into activation mechanism and product release. J. Struct. Biol. **187**: 215–222. doi:10.1016/j.jsb.2014.07.007. PMID:25108239.

Haba, E., Bresco, O., Ferrer, C., Marqués, A., Busquets, M., and Manresa, A. 2000. Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. Enzyme Microb. Technol. **26**: 40–44. doi:10.1016/S0141-0229(99)00125-8.

Haki, G.D., and Rakshit, S.K. 2003. Developments in industrially important thermostable enzymes: a review. Bioresour. Technol. **89**: 17–34. doi:10.1016/S0960-8524(03)00033-6. PMID:12676497.

Hasan, F., Shah, A.A., and Hameed, A. 2006. Industrial applications of microbial lipases. Enzyme Microb. Technol. **39**: 235–251. doi:10.1016/j.enzmictec.2005.10.016.

Hatzakis, N.S., Daphnomili, D., and Smonou, I. 2003. Ferulic acid esterase from *Humicola insolens* catalyzes enantioselective transesterification of secondary alcohols. J. Mol. Catal. B: Enzym. **21**: 309–311. doi:10.1016/S1381-1177(02)00228-X.

Hausmann, S., and Jaeger, K.E. 2010. Lipolytic enzymes from bacteria. *In* Handbook of hydrocarbon and lipid microbiology. Vol. 2. *Edited by* K.N. Timmis, T.J. McGenity, J.R. van der Meer, and V. de Lorenzo. Springer, Berlin, Germany. pp. 1099–1126.

Henderson, I.R., Navarro-Garcia, F., and Nataro, J.P. 1998. The great escape: structure and function of the autotransporter proteins. Trends Microbiol. **6**: 370–378. doi:10.1016/S0966-842X(98)01318-3. PMID:9778731.

Henne, A., Schmitz, R.A., Bömeke, M., Gottschalk, G., and Daniel, R. 2000. Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. Appl. Environ. Microbiol. **66**: 3113–3116. doi:10.1128/AEM.66.7.3113-3116.2000. PMID:10877816.

Heravi, K.M., Yakhchali, B., Eftekhar, F., Vafadar-Isfahani, B., Ghomi, H.H., and Ahmadi-Danesh, H. 2009. Molecular cloning and characterization of a lipase from an indigenous *Bacillus pumilus*. J. Sci., Islamic Repub. Iran, **20**: 213–220.

Hong, K.H., Jang, W.H., Choi, K.D., and Yoo, O.J. 1991. Characterization of *Pseudomonas fluorescens* carboxyl esterase: cloning and expression of the esterase gene in *Escherichia coli*. Agric. Biol. Chem. **55**: 2839–2845. doi:10.1271/bbb1961.55.2839. PMID:1368750.

Horne, I., Harcourt, R.L., Sutherland, T.D., Russell, R.J., and Oakeshott, J.G. 2002. Isolation of a *Pseudomonas monteilli* strain with a novel phosphotriesterase. FEMS Microbiol. Lett. **206**: 51–55. doi:10.1111/j.1574-6968.2002.tb10985.x. PMID:11786256.

Hotta, Y., Ezaki, S., Atomi, H., and Imanaka, T. 2002. Extremely stable and versatile carboxylesterase from a hyperthermophilic archaeon. Appl. Environ. Microbiol. **68**: 3925–3931. doi:10.1128/AEM.68.8.3925-3931.2002. PMID:12147492.

Huang, Y.-C., Chen, G.-H., Chen, Y.-F., Chen, W.-L., and Yang, C.-H. 2010. Heterologous expression of thermostable acetylxylan esterase gene from *Thermobifida fusca* and its synergistic action with xylanase for the production of xylooligosaccharides. Biochem. Biophys. Res. Commun. **400**: 718–723. doi:10.1016/j.bbrc.2010.08.136. PMID:20816933.

Hudlicky, T., and Reed, J.W. 2009. Applications of biotransformations and biocatalysis to complexity generation in organic synthesis. Chem. Soc. Rev. **38**: 3117–3132. doi:10.1039/b901172m. PMID:19847346.

Imran, M., Asad, M.J., Hadri, S.H., and Mehmood, S. 2012. Production and industrial applications of laccase enzyme. J. Cell Mol. Biol. **10**: 1–11.

Jacoby, G.A. 2009. AmpC β-lactamases. Clin. Microbiol. Rev. **22**: 161–182. doi:10.1128/CMR.00036-08.

Jaeger, K.E., and Eggert, T. 2002. Lipases for biotechnology. Curr. Opin. Biotechnol. **13**: 390–397. doi:10.1016/S0958-1669(02)00341-5. PMID:12323363.

Jaeger, K.E., and Reetz, M.T. 1998. Microbial lipases form versatile tools for biotechnology. Trends Biotechnol. **16**: 396–403. doi:10.1016/S0167-7799(98)01195-0. PMID:9744114.

Jaeger, K.E., Dijkstra, B.W., and Reetz, M.T. 1999. Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu. Rev. Microbiol. **53**: 315–351. doi:10.1146/annurev.micro.53.1.315. PMID:10547694.

Jegannathan, K.R., and Nielsen, P.H. 2013. Environmental assessment of enzyme use in industrial production — a literature review. J. Cleaner Prod. **42**: 228–240. doi:10.1016/j.jclepro.2012.11.005.

Kamini, N.R., Fujii, T., Kurosu, T., and Iefuji, H. 2000. Production, purification and characterization of an extracellular lipase from the yeast, *Cryptococcus* sp. S-2. Process Biochem. **36**: 317–324. doi:10.1016/S0032-9592(00)00228-4.

Kanaya, S., Koyanagi, T., and Kanaya, E. 1998. An esterase from *Escherichia coli* with a sequence similarity to hormone-sensitive lipase. Biochem. J. **332**: 75–80. doi:10.1042/bj3320075. PMID:9576853.

Kang, C.-H., Oh, K.-H., Lee, M.-H., Oh, T.-K., Kim, B.H., and Yoon, J.-H. 2011. A novel family VII esterase with industrial potential from compost metagenomic library. Microb. Cell Fact. **10**: 41–49. doi:10.1186/1475-2859-10-41. PMID:21619698.

Khalameyzer, V., Fischer, I., Bornscheuer, U.T., and Altenbuchner, J. 1999. Screening, nucleotide sequence, and biochemical characterization of an esterase from *Pseudomonas fluorescens* with highly activity towards lactones. Appl. Environ. Microbiol. **65**: 477–482. PMID:9925571.

Kim, E.Y., Oh, K.H., Lee, M.H., Kang, C.H., Oh, T.K., and Yoon, J.H. 2009. Novel cold-adapted alkaline lipase from an intertidal flat metagenome and proposal for a new family of bacterial lipases. Appl. Environ. Microbiol. **75**: 257–260. doi:10.1128/AEM.01400-08. PMID:18931297.

Kim, K.K., Song, H.K., Shin, D.H., Hwang, K.Y., Choe, S., Yoo, O.J., and Suh, S.W. 1997. Crystal structure of carboxylesterase from *Pseudomonas fluorescens*, an α/β hydrolase with broad substrate specificity. Structure, **5**: 1571–1584. doi:10.1016/S0969-2126(97)00306-7. PMID:9438866.

Kim, Y.S., Lee, H.B., Choi, K.D., Park, S., and Yoo, O.J. 1994. Cloning of *Pseudomonas fluorescens* carboxylesterase gene and characterization of its product expressed in *Escherichia coli*. Biosci. Biotechnol. Biochem. **58**: 111–116. doi:10.1271/bbb.58.111. PMID:7764506.

Kim, Y.-J., Choi, G.-S., Kim, S.-B., Yoon, G.-S., Kim, Y.-S., and Ryu, Y.-W. 2006. Screening and characterization of a novel esterase from a metagenomic library. Protein Expression Purif. **45**: 315–323. doi:10.1016/j.pep.2005.06.008.

Kirk, O., Borchert, T.V., and Fuglsang, C.C. 2002. Industrial enzyme applications. Curr. Opin. Biotechnol. **13**: 345–351. doi:10.1016/S0958-1669(02)00328-2. PMID:12323357.

Knapp, A., Voget, S., Gao, R., Zaburannyi, N., Krysciak, D., Breuer, M., et al. 2016. Mutations improving production and secretion of extracellular lipase by *Burkholderia glumae* PG1. Appl. Microbiol. Biotechnol. **100**: 1265–1273. doi:10.1007/s00253-015-7041-z.

Kojima, Y., Yokoe, M., and Mase, T. 1994. Purification and characterization of an alkaline lipase from *Pseudomonas fluorescens* AK102. Biosci., Biotechnol., Biochem. **58**: 1564–1568. doi:10.1271/bbb.58.1564. PMID:7765474.

Kontkanen, H., Tenkanen, M., Fagerström, R., and Reinikainen, T. 2004. Characterisation of steryl esterase activities in commercial lipase preparations. J. Biotechnol. **108**: 51–59. doi:10.1016/j.jbiotec.2003.11.003. PMID:14741769.

Kumar, D., Kumar, L., Nagar, S., Raina, C., Parshad, R., and Gupta, V.K. 2012. Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain DVL2 and its potential evaluation in esterification and resolution reactions. Arch. Appl. Sci. Res. **4**: 1763–1770.

Lailaja, V.P., and Chandrasekaran, M. 2013. Detergent compatible alkaline lipase produced by marine *Bacillus smithii* BTMS 11. World J. Microbiol. Biotechnol. **29**: 1349–1360. doi:10.1007/s11274-013-1298-0. PMID:23443894.

Langin, D., Laurell, H., Holst, L.S., Belfrage, P., and Holm, C. 1993. Gene organization and primary structure of human hormone-sensitive lipase: possible significance of a sequence homology with a lipase of *Moraxella* TA144, an Antarctic bac-

terium. Proc. Natl. Acad. Sci. U.S.A. **90**: 4897–4901. doi:10.1073/pnas.90.11.4897. PMID:8506334.

Lee, M., Lee, C., Oh, T., Song, J.K., and Yoon, J. 2006. Isolation and characterization of a novel lipase from a metagenomic library of tidal flat sediments: evidence for a new family of bacterial lipases. Appl. Environ. Microb. **72**: 7406–7409. doi:10.1128/AEM.01157-06.

Lee, Y.-S. 2016. Isolation and characterization of a novel cold-adapted esterase, MtEst45, from *Microbulbifer thermotolerans* DAU221. Front. Microbiol. **7**: 218–231. PMID:26973604.

Li, S., Yang, X., Yang, S., Zhu, M., and Wang, X. 2012. Technology prospecting on enzymes: application, marketing and engineering. Comput. Struct. Biotechnol. J. **2**: e201209017. PMID:24688658.

Li, X.-L., Zhang, W.-H., Wang, Y.-D., Dai, Y.-J., Zhang, H.-T., Wang, Y., et al. 2014. A high-detergent-performance, cold-adapted lipase from *Pseudomonas stutzeri* PS59 suitable for detergent formulation. J. Mol. Catal. B: Enzym. **102**: 16–24. doi:10.1016/j.molcatb.2014.01.006.

Liaw, R.B., Cheng, M.P., Wu, M.C., and Lee, C.Y. 2010. Use of metagenomic approaches to isolate lipolytic genes from activated sludge. Bioresour. Technol. **101**: 8323–8329. doi:10.1016/j.biortech.2010.05.091. PMID:20639117.

Lima, L.N., Oliveira, G.C., Rojas, M.J., Castro, H.F., Da Rós, P.C.M., Mendes, A.A., et al. 2015. Immobilization of *Pseudomonas fluorescens* lipase on hydrophobic supports and application in biodiesel synthesis by transesterification of vegetable oils in solvent-free systems. J. Ind. Microbiol. Biotechnol. **42**: 523–535. doi:10.1007/s10295-015-1586-9. PMID:25626526.

Litthauer, D., Ginster, A., and van Eeden Skein, E. 2002. *Pseudomonas luteola* lipase: a new member of the 320-residue *Pseudomonas* lipase family. Enzyme Microb. Technol. **30**: 209–215. doi:10.1016/S0141-0229(01)00469-0.

Liu, T.Z., Wang, D.F., Zhao, H.Y., and Zheng, K.Y. 2012. Stickies treatments of recycled fiber pulping wastewater by lipases. Adv. Mater. Res. **534**: 225–229. doi:10.4028/www.scientific.net/AMR.534.225.

Lobkovsky, E., Moews, P.C., Liu, H., Zhao, H., Frere, J.M., and Knox, J.R. 1993. Evolution of an enzyme activity: Crystallographic structure at 2-A resolution of cephalosporinase from the *ampC* gene of *Enterobacter cloacae* P99 and comparison with a class A penicillinase. Proc. Natl. Acad. Sci. U.S.A. **90**: 11257–11261. doi:10.1073/pnas.90.23.11257. PMID:8248237.

Lomolino, G., Rizzi, C., Spettoli, P., Curioni, A., and Lante, A. 2003. Cell vitality and esterase activity of *Saccharomyces cerevisiae* is affected by increasing calcium concentration. Agro Food Industry Hi Tech, **14**(6): 32–35.

Loveless, B.J., and Saier, M.H., Jr. 1997. A novel family of channel-forming, autotransporting, bacterial virulence factors. Mol. Membr. Biol. **14**: 113–123. doi:10.3109/09687689709048171. PMID:9394291.

Lu, Y., Lin, Q., Wang, J., Wu, Y., Bao, W., Lv, F., and Lu, Z. 2010. Overexpression and characterization in Bacillus subtilis of a positionally nonspecific lipase from *Proteus vulgaris*. J. Ind. Microbiol. Biotechnol. **37**: 919–925. doi:10.1007/s10295-010-0739-0. PMID:20490605.

Manco, G., Giosuè, E., D'Auria, S., Herman, P., Carrea, G., and Rossi, M. 2000. Cloning, overexpression, and properties of a new thermophilic and thermostable esterase with sequence similarity to hormone-sensitive lipase subfamily from the archaeon *Archaeoglobus fulgidus*. Arch. Biochem. Biophys. **373**: 182–192. doi:10.1006/abbi.1999.1497. PMID:10620337.

Manco, G., Mandrich, L., and Rossi, M. 2001. Residues at the active site of the esterase 2 from *Alicyclobacillus acidocaldarius* involved in substrate specificity and catalytic activity at high temperature. J. Biol. Chem. **276**: 37482–37490. doi:10.1074/jbc.M103017200. PMID:11447219.

Margesin, R., Feller, G., Gerday, C., and Russell, N.J. 2002. Cold-adapted micro-organisms: adaptation strategies and biotechnological potential. *In* Encyclopedia of environmental microbiology. *Edited by* G. Bitton. Wiley, N.Y., USA. pp. 871–885. doi:10.1002/0471263397.env150.

Massadeh, M.I., and Sabra, F.M. 2011. Production and characterization of lipase from *Bacillus stearothermophilus*. Afr. J. Biotechnol. **10**: 13139–13146.

Messaoudi, A., Belguith, H., Ghram, I., and Hamida, J.B. 2011. LIPABASE: a database for 'true' lipase family enzymes. Int. J. Bioinf. Res. Appl. **7**: 390–401. doi:10.1504/IJBRA.2011.043770. PMID:22112530.

Metin, K., Burcu Bakir Ateslier, Z., Basbulbul, G., and Halil Biyik, H. 2006. Characterization of esterase activity in *Geobacillus* sp. HBB-4. J. Basic Microbiol. **46**: 400–409. doi:10.1002/jobm.200510121. PMID:17009295.

Mohamed, Y.M., Ghazy, M.A., Sayed, A., Ouf, A., El-Dorry, H., and Siam, R. 2013. Isolation and characterization of a heavy metal-resistant, thermophilic esterase from a Red Sea brine pool. Sci. Rep. **3**: 3358–3366. PMID:24285146.

Moukouli, M., Topakas, E., and Christakopoulos, P. 2008. Cloning, characterization and functional expression of an alkali-tolerant type C feruloyl esterase from *Fusarium oxysporum*. Appl. Microbiol. Biotechnol. **79**: 245–254. doi:10.1007/s00253-008-1432-3. PMID:18414848.

Moynihan, P.J., and Clarke, A.J. 2010. *O*-Acetylation of peptidoglycan in gram-negative bacteria. Identification and characterization of peptidoglycan *O*-acetyltransferase in *Neisseria gonorrhoeae*. J. Biol. Chem. **23**: 13264–13273. doi:10.1074/jbc.M110.107086.

Nacke, H., Will, C., Herzog, S., Nowka, B., Engelhaupt, M., and Daniel, R. 2011. Identification of novel lipolytic genes and gene families by screening of metagenomic libraries derived from soil samples of the German Biodiversity Exploratories. FEMS Microbiol. Ecol. **78**: 188–201. doi:10.1111/j.1574-6941.2011.01088.x. PMID:21395625.

Nerurkar, M., Joshi, M., Pariti, S., and Adivarekar, R. 2013. Application of lipase from marine bacteria *Bacillus sonorensis* as an additive in detergent formulation. J. Surfactants Deterg. **16**: 435–443. doi:10.1007/s11743-012-1434-0.

Ngo, T.D., Ryu, B.H., Ju, H., Jang, E., Park, K., Kim, K.K., and Kim, T.D. 2013. Structural and functional analyses of a bacterial homologue of hormone-sensitive lipase from a metagenomic library. Acta Crystallogr., Sect D: Biol. Crystallogr. **69**: 1726–1737. doi:10.1107/S0907444913013425.

Nigam, P.S. 2013. Microbial enzymes with special characteristics for biotechnological applications. Biomolecules, **3**: 597–611. doi:10.3390/biom3030597. PMID:24970183.

Nishimura, M., and Inouye, S. 2000. Inhibitory effects of carbohydrates on cholesterol esterase biosynthesis in *Streptomyces lavendulae* H646-SY2. J. Biosci. Bioeng. **90**: 564–566. doi:10.1016/S1389-1723(01)80042-6. PMID:16232911.

Nishizawa, M., Shimizu, M., Ohkawa, H., and Kanaoka, M. 1995. Stereoselective production of (+)-*trans*-chrysanthemic acid by a microbial esterase: cloning, nucleotide sequence, and overexpression of the esterase gene of *Arthrobacter globiformis* in *Escherichia coli*. Appl. Environ. Microbiol. **61**: 3208–3215. PMID:7574629.

Ogierman, M.A., Fallarino, A., Riess, T., Williams, S.G., Attridge, S.R., and Manning, P.A. 1997. Characterization of the *Vibrio cholerae* El Tor lipase operon *lipAB* and a protease gene downstream of the *hly* region. J. Bacteriol. **179**: 7072–7080. doi:10.1128/jb.179.22.7072-7080.1997. PMID:9371455.

Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., et al. 1992. The α/β hydrolase fold. Protein Eng., Des. Sel. **5**: 197–211. doi:10.1093/protein/5.3.197. PMID:1409539.

Østerlund, T., Danielsson, B., Degerman, E., Contreras, J.A.,

Edgren, G., Davis, R.C., et al. 1996. Domain-structure analysis of recombinant rat hormone-sensitive lipase. Biochem. J. **319**: 411–420. doi:10.1042/bj3190411. PMID:8912675.

Patel, R.N. 2000. Stereoselective biocatalysts. Dekker, N.Y., USA.

Patel, V., Nambiar, S., and Madamwar, D. 2014. An extracellular solvent stable alkaline lipase from *Pseudomonas* sp. DMVR46: partial purification, characterization and application in non-aqueous environment. Process Biochem. **49**: 1673–1681. doi:10.1016/j.procbio.2014.06.007.

Patrick, K. 2004. Enzyme technology improves efficiency, cost, safety of stickies removal program. Paper Age, **September**: 22–25.

Petersen, E.I., Valinger, G., Sölkner, B., Stubenrauch, G., and Schwab, H. 2001. A novel esterase from *Burkholderia gladioli* which shows high deacetylation activity on cephalosporins is related to β-lactamases and DD-peptidases. J. Biotechnol. **89**: 11–25. doi:10.1016/S0168-1656(01)00284-X. PMID:11472796.

Pleiss, J., Fischer, M., and Schmid, R.D. 1998. Anatomy of lipase binding sites: the scissile fatty acid binding site. Chem. Phys. Lipids, **93**: 67–80. doi:10.1016/S0009-3084(98)00030-9. PMID:9720251.

Pohlenz, H.D., Boidol, W., Schüttke, I., and Streber, W.R. 1992. Purification and properties of an *Arthrobacter oxydans* P52 carbamate hydrolase specific for the herbicide phenmedipham and nucleotide sequence of the corresponding gene. J. Bacteriol. **174**: 6600–6607. doi:10.1128/jb.174.20.6600-6607.1992. PMID:1400211.

Prasad, M.P. 2014. Production of lipase enzyme from *Pseudomonas aeruginosa* isolated from lipid rich soil. Int. J. Pure Appl. Biosci. **2**: 77–81.

Prim, N., Bofill, C., Pastor, F.I.J., and Diaz, P. 2006. Esterase EstA6 from *Pseudomonas* sp. CR-611 is a novel member in the utmost conserved cluster of family VI bacterial lipolytic enzymes. Biochimie, **88**: 859–867. doi:10.1016/j.biochi.2006.02.011. PMID:16600467.

Pringle, D., and Dickstein, R. 2004. Purification of ENOD8 proteins from *Medicago sativa* root nodules and their characterization as esterases. Plant Physiol. Biochem. **42**: 73–79. doi:10.1016/j.plaphy.2003.10.004. PMID:15061087.

Quyen, T.D., Vu, C.H., and Le, G.T. 2012. Enhancing functional production of a chaperone-dependent lipase in *Escherichia coli* using the dual expression cassette plasmid. Microb. Cell Fact. **11**: 29. doi:10.1186/1475-2859-11-29. PMID:22380513.

Ramteke, P.W., Joseph, B., and Kuddus, M. 2005. Extracellular lipases from anaerobic microorganisms of Antarctic. Ind. J. Biotech. **4**: 293–294.

Rashid, N., Shimada, Y., Ezaki, S., Atomi, H., and Imanaka, T. 2001. Low-temperature lipase from psychrotrophic *Pseudomonas* sp. strain KB700A. Appl. Environ. Microbiol. **67**: 4064–4069. doi:10.1128/AEM.67.9.4064-4069.2001. PMID:11526006.

Ro, H.-S., Hong, H.P., Kho, B.H., Kim, S., and Chung, B.H. 2004. Genome-wide cloning and characterization of microbial esterases. FEMS Microbiol. Lett. **233**: 97–105. doi:10.1016/j.femsle.2004.01.046. PMID:15043875.

Rodrigues, J., Canet, A., Rivera, I., Osório, N.M., Sandoval, G., Valero, F., and Ferreira-Dias, S. 2016. Biodiesel production from crude Jatropha oil catalysed by non-commercial immobilized heterologous *Rhizopus oryzae* and *Carica papaya* lipases. Bioresour. Technol. **213**: 88–95. doi:10.1016/j.biortech.2016.03.011. PMID:26980626.

Rosenau, F., and Jaeger, K.-E. 2000. Bacterial lipases from *Pseudomonas*: regulation of gene expression and mechanisms of secretion. Biochimie, **82**: 1023–1032. doi:10.1016/S0300-9084(00)01182-2. PMID:11099799.

Rosenau, F., Tommassen, J., and Jaeger, K.E. 2004. Lipase-specific foldases. ChemBioChem, **5**: 152–161. doi:10.1002/cbic.200300761. PMID:14760735.

Rúa, M.L., Díaz-Mauriño, T., Fernández, V.M., Otero, C., and Ballesteros, A. 1993. Purification and characterization of two distinct lipases from *Candida cylindracea*. Biochim. Biophys. Acta, Gen. Subj. **1156**: 181–189. doi:10.1016/0304-4165(93)90134-T. PMID:8427877.

Ruiz, C., Falcocchio, S., Pastor, F.I.J., Saso, L., and Diaz, P. 2007. *Helicobacter pylori* EstV: identification, cloning, and characterization of the first lipase isolated from an epsilon-proteobacterium. Appl. Environ. Microbiol. **73**: 2423–2431. doi:10.1128/AEM.02215-06. PMID:17293528.

Santarossa, G., Lafranconi, P.G., Alquati, C., DeGioia, L., Alberghina, L., Fantucci, P., and Lotti, M. 2005. Mutations in the "lid" region affect chain length specificity and thermostability of a *Pseudomonas fragi* lipase. FEBS Lett. **579**: 2383–2386. doi:10.1016/j.febslet.2005.03.037. PMID:15848176.

Saranya, P., Kumari, H.S., Rao, B.P., and Sekaran, G. 2014. Lipase production from a novel thermo-tolerant and extreme acidophile *Bacillus pumilus* using palm oil as the substrate and treatment of palm oil-containing wastewater. Environ. Sci. Pollut. Res. **21**: 3907–3919. doi:10.1007/s11356-013-2354-x.

Sarrouh, B., Santos, T.M., Miyoshi, A., Dias, R., and Azevedo, V. 2012. Up-to-date insight on industrial enzymes applications and global market. J. Bioprocess. Biotech. **S4**: 002. doi:10.4172/2155-9821.S4-002.

Sasso, F., Natalello, A., Castoldi, S., Lotti, M., Santambrogio, C., and Grandori, R. 2016. *Burkholderia cepacia* lipase is a promising biocatalyst for biofuel production. Biotechnol. J. **11**: 954–960. doi:10.1002/biot.201500305. PMID:27067648.

Schmidt-Dannert, C., Sztajer, H., Stöcklein, W., Menge, U., and Schmid, R.D. 1994. Screening, purification and properties of a thermophilic lipase from *Bacillus thermocatenulatus*. Biochim. Biophys. Acta, Lipids Lipid Metab. **1214**: 43–53. doi:10.1016/0005-2760(94)90008-6.

Schmiel, D.H., and Miller, V.L. 1999. Bacterial phospholipases and pathogenesis. Microbes Infect. **1**: 1103–1112. doi:10.1016/S1286-4579(99)00205-1. PMID:10572314.

Shao, W., and Wiegel, J. 1995. Purification and characterization of two thermostable acetyl xylan esterases from *Thermoanaerobacterium* sp. strain JW/SL-YS485. Appl. Environ. Microbiol. **61**: 729–733. PMID:7574610.

Sharma, R., Chisti, Y., and Banerjee, U.C. 2001. Production, purification, characterization, and applications of lipases. Biotechnol. Adv. **19**: 627–662. doi:10.1016/S0734-9750(01)00086-6. PMID:14550014.

Sharma, S., and Kanwar, S.S. 2014. Organic solvent tolerant lipases and applications. Sci. World J. **2014**: 625258. doi:10.1155/2014/625258.

Sharon, C., Furugoh, S., Yamakido, T., Ogawa, H.I., and Kato, Y. 1998. Purification and characterization of a lipase from *Pseudomonas aeruginosa* KKA-5 and its role in castor oil hydrolysis. J. Ind. Microbiol. Biotechnol. **20**: 304–307. doi:10.1038/sj.jim.2900528.

Shruthi, H., Babu, M.M., and Sankaran, K. 2010. TAT-pathway-dependent lipoproteins as a niche-based adaptation in prokaryotes. J. Mol. Evol. doi:10.1007/s00239-010-9334-2.

Shu, Z., Wu, J., Chen, D., Cheng, L., Zheng, Y., Chen, J., and Huang, J. 2016. Optimization of *Burkholderia* sp. ZYB002 lipase production for pitch control in thermomechanical pulping (TMP) processes. Holzforschung, **66**: 341–348. doi:10.1515/hf.2011.152.

Skals, P.B., Krabek, A., Nielsen, P.H., and Wenzel, H. 2008. Environmental assessment of enzyme assisted processing in pulp and paper industry. Int. J. Life Cycle Assess. **13**: 124–132. doi:10.1065/lca2007.11.366.

Sørensen, H.P., and Mortensen, K.K. 2005. Soluble expression of recombinant proteins in the cytoplasm of *Escherichia coli*. Microb. Cell Fact. **4**: 1. doi:10.1186/1475-2859-4-1.

Speranza, P., Ribeiro, A.P.B., and Macedo, G.A. 2016. Application of lipases to regiospecific interesterification of exotic oils

from an Amazonian area. J. Biotechnol. **218**: 13–20. doi:10.1016/j.jbiotec.2015.11.025. PMID:26657709.

Sugihara, A., Shimada, Y., Nomura, A., Terai, T., Imayasu, M., Nagai, Y., et al. 2002. Purification and characterization of a novel cholesterol esterase from *Pseudomonas aeruginosa*, with its application to cleaning lipid-stained contact lenses. Biosci. Biotechnol. Biochem. **66**: 2347–2355. doi:10.1271/bbb.66.2347. PMID:12506971.

Suzuki, T., Nakayama, T., Choo, D.W., Hirano, Y., Kurihara, T., Nishino, T., and Esaki, N. 2003. Cloning, heterologous expression, renaturation, and characterization of a cold-adapted esterase with unique primary structure from a psychrotroph *Pseudomonas* sp. strain B11-1. Protein Expression Purif. **30**: 171–178. doi:10.1016/S1046-5928(03)00128-1.

Svendsen, A., Borch, K., Barfoed, M., Nielsen, T.B., Gormsen, E., and Patkar, S.A. 1995. Biochemical properties of cloned lipases from the *Pseudomonas* family. Biochim. Biophys. Acta, Lipids Lipid Metab. **1259**: 9–17. doi:10.1016/0005-2760(95)00117-U.

Terahara, T., Yamada, K., Kurata, S., Yokomaku, T., Tsuneda, S., and Harayama, S. 2010. Direct cloning and expression of putative esterase genes from environmental DNA. Enzyme Microb. Technol. **47**: 17–23. doi:10.1016/j.enzmictec.2010.04.005.

Tielen, P., Kuhn, H., Rosenau, F., Jaeger, K.-E., Flemming, H.-C., and Wingender, J. 2013. Interaction between extracellular lipase LipA and the polysaccharide alginate of *Pseudomonas aeruginosa*. BMC Microbiol. **13**: 159–171. doi:10.1186/1471-2180-13-159. PMID:23848942.

Tjalsma, H., Bolhuis, A., Jongbloed, J.D.H., Bron, S., and van Dijl, J.M. 2000. Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. Microbiol. Mol. Biol. Rev. **64**: 515–547. doi:10.1128/MMBR.64.3.515-547.2000. PMID:10974125.

Topakas, E., Kalogeris, E., Kekos, D., Macris, B.J., and Christakopoulos, P. 2003. Production and partial characterisation of feruloyl esterase by *Sporotrichum thermophile* in solid-state fermentation. Process Biochem. **38**: 1539–1543. doi:10.1016/S0032-9592(03)00044-X.

Tserovska, L., Dimkov, R., Rasheva, T., and Yordanova, T. 2006. Extra- and intracellular esterases involved in dimethylterephthalate catabolism by *Pseudomonas* sp. J. Culture Collections, **5**: 35–37.

Upton, C., and Buckley, J.T. 1995. A new family of lipolytic enzymes? Trends Biochem. Sci. **20**: 178–179. doi:10.1016/S0968-0004(00)89002-7. PMID:7610479.

Uwajima, T., and Terada, O. 1976. Purification and properties of cholesterol esterase from *Pseudomonas fluorescens*. Agric. Biol. Chem. **40**: 1957–1964. doi:10.1080/00021369.1976.10862345.

van Tilbeurgh, H., Egloff, M.-P., Martinez, C., Rugani, N., Verger, R., and Cambillau, C. 1993. Interfacial activation of the lipase-procolipase complex by mixed micelles revealed by X-ray crystallography. Nature, **362**: 814–820. doi:10.1038/362814a0. PMID:8479519.

Vaquero, M.E., Barriuso, J., Medrano, F.J., Prieto, A., and Martinez, M.J. 2015. Heterologous expression of a fungal sterol esterase/lipase in different hosts: effect on solubility, glycosylation and production. J. Biosci. Bioeng. **120**: 637–643. doi:10.1016/j.jbiosc.2015.04.005. PMID:25939548.

Verger, R. 1997. 'Interfacial activation' of lipases: facts and artifacts. Trends Biotechnol. **15**: 32–38. doi:10.1016/S0167-7799(96)10064-0.

Vijayalakshmi, S., Venkatkumar, S., and Thankamani, V. 2011. Screening of alkalophilic thermophilic protease isolated from *Bacillus* RV.B2.90 for industrial applications. Res. Biotechnol. **2**: 32–41.

Villeneuve, P., Muderhwa, J.M., Graille, J., and Haas, M.J. 2000. Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches. J. Mol. Catal. B Enzym. **9**: 113–148. doi:10.1016/S1381-1177(99)00107-1.

Whangsuk, W., Sungkeeree, P., Thiengmag, S., Kerdwong, J., Sallabhan, R., Mongkolsuk, S., and Loprasert, S. 2013. Gene cloning and characterization of a novel highly organic solvent tolerant lipase from *Proteus* sp. SW1 and its application for biodiesel production. Mol. Biotechnol. **53**: 55–62. PMID:22371263.

Wu, X., You, P., Su, E., Xu, J., Gao, B., and Wei, D. 2012. In vivo functional expression of a screened *P. aeruginosa* chaperone-dependent lipase in *E. coli*. BMC Biotechnol. **12**: 58. doi:10.1186/1472-6750-12-58.

Xun, E.-N., Lv, X.-L., Kang, W., Wang, J.-X., Zhang, H., Wand, L., and Wang, Z. 2012. Immobilization of *Pseudomonas fluorescens* lipase onto magnetic nanoparticles for resolution of 2-octanol. Appl. Biochem. Biotechnol. **168**: 697–707. doi:10.1007/s12010-012-9810-9. PMID:22847187.

Yang, J.L., Ma, J., Pierce, J.M., and Eriksson, K.-E.L. 1994. Composition for enzymatic deinking of waste paper. Patent US6767728 B2.

Zhang, Y., Wu, J., Miao, Q., Chen, L., Luo, X., and Huang, L. 2013. Lipase-aided deinking of mixed office waste paper. J. Fujian Agric. For. Univ. (Natural Science Edition), 2013-01.

Zhao, L.-L., Xu, J.-H., Zhao, J., Pan, J., and Wang, Z.-L. 2008. Biochemical properties and potential applications of an organic solvent-tolerant lipase isolated from *Serratia marcescens* ECU1010. Process Biochem. **43**: 626–633. doi:10.1016/j.procbio.2008.01.023.

Zock, J., Cantwell, C., Swartling, J., Hodges, R., Pohl, T., Sutton, K., et al. 1994. The *Bacillus subtilis pnbA* gene encoding *p*-nitrobenzyl esterase: cloning, sequence and high-level expression in *Escherichia coli*. Gene, **151**: 37–43. doi:10.1016/0378-1119(94)90630-0. PMID:7828905.