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# Bacterial lipases: A review on purification and characterization

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ABSTRACT

Lipase (E.C.3.1.1.3) belongs to the hydrolases and is also known as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase. Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids in an oil-water interface. These are widely used in food, dairy, flavor, pharmaceuticals, biofuels, leather, cosmetics, detergent, and chemical industries. Lipases are of plant, animal, and microbial origin, but microbial lipases are produced at industrial level and represent the most widely used class of enzymes in biotechnological applications and organic chemistry. Phylogenetic analysis and comparison of residues around GxSxG motif provided an insight to the diversity among bacterial lipases. A variety of para-Nitrophenyl (p-NP) esters having $C_2$ to $C_{16}$ (p-NP acetate to p-NP palmitate) in their fatty acid side chain can be hydrolyzed by bacterial lipases. Large heterogeneity has been observed in molecular and catalytic characteristics of lipases including molecular mass; 19–96 kDa, $K_m$; 0.0064 –16.58 mM, $K_{cat}$; 0.1665–1.0 × $10^4$ $s^{-1}$ and $K_{cat}/K_m$; 26.02–7377 $s^{-1}$/mM. Optimal conditions of their working temperature and pH have been stated 15–70 °C and 5.0–10.8, respectively and are strongly associated with the type and growth conditions of bacteria. Surface hydrophobicity, enzyme activity, stability in organic solvents and at high temperature, proteolytic resistance and substrate tolerance are the properties of bacterial lipases that have been improved by engineering. Bacterial lipases have been extensively studied during last decade. However, their wider applications demand a detailed review on purification, catalytic characterization and applications of lipases.



**Contents**



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## 1. Introduction

Significant rising concern in the field of enzymology on account of wider applications of enzymes in various chemical processes has been increased since a few decades (Pliego et al., 2015). Due to versatile applications, lipases are the third most abundantly used enzymes after proteases and amylases (carbohydrases) (Ülker et al., 2011). Lipase (E.C.3.1.1.3) is also known as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase and belongs to the class of enzymes that catalyze the hydrolysis reactions (hydrolases). Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids in an oil-water interface. Lipases also have a property to reverse this reaction in an aqueous and non-aqueous media (Faouzi et al., 2015b; Lee et al., 2015; Priji et al., 2015; Ramos-Sanchez et al., 2015; Ullah et al., 2015). Some lipases show enantioselective properties and used to catalyze the processes of esterification, interesterification, transesterification, acidolysis and aminolysis (Hasan et al., 2009). The substrates of lipases (long chain triacylglycerols) are insoluble in water, hence, these are first dissolved in organic solvents followed by mixing with buffer (two-phase system). However, lipases are soluble in water and can catalyze their reactions in two types of systems including aqueous and organic medium. Organic solvents may denature and cause conformational changes in the lipase structure and hence influence their functional and catalytic activities (Guo et al., 2015).

Lipases are ubiquitous enzymes (Priji et al., 2015), belong to α/β hydrolase fold super-family (Kapoor and Gupta, 2012) and have a network of hydrogen bonds at their active site containing triad of Ser, Asp (Glu) and His (Faouzi et al., 2015b; Farrokh et al., 2014; Thakur et al., 2014). Lipase catalyzed transesterification for the production of biodiesel is an efficient, energy-saving, and environment friendly process and is a promising alternative to the conventional chemical catalysis (Fjerbaek et al., 2009). Lipases are substrate specific enzymes and have properties like chemo-, region-, stereo-specificity and ability to catalyze heterogeneous reactions both in water soluble and water insoluble systems. On account of their wider catalytic properties, lipases are extensively used as biocatalysts in different industries like agrochemical, pharmaceutical, detergent, tanning, food and surfactant producing industries (Ananthi et al., 2014; Iftikhar et al., 2012; Kumar et al., 2012a,b; Thakur et al., 2014).

On the basis of positional specificity (regiospecificity), lipases are divided into three classes.

*(i). Non-specific lipases*

These lipases catalyze the triglyceride into free fatty acids and glycerol with mono- and di-glycerides as intermediates and can remove fatty acid from any position of the substrate. Mono- and di-glycerides are hydrolyzed more rapidly than triglyceride. (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

*(ii). 1, 3-specific lipases*

These lipases release fatty acids from position 1 and 3 of the triglycerides and cannot hydrolyze ester bonds at secondary positions. Hydrolysis of triglycerides by 1, 3-specific lipases to diglycerides is much faster than those into mono glycerides (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

*(iii). Fatty acid-specific lipases*

A third group of lipases shows fatty acid selectivity and catalyzes the hydrolysis of esters which have long-chain fatty acids with double bonds in cis position between C-9 and C-10 (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

Lipases need no co-factor for their activity and remain active in organic solvents (Lee et al., 2015; Ullah et al., 2015). Consumption of all monoglycerides, diglycerides, triglycerides and free fatty acids in the process of transesterification, high production in non-aqueous media, low reaction time and resistance to low pH are some of the properties which make lipases more desirable biocatalysts (Ashfaq, 2015). Lipases are of plant, animal, and microbial origin, but microbial lipases are produced at industrial level and represent the most widely used class of enzymes in biotechnological applications and organic chemistry due to higher catalytic activity, seasonal changes independent production, ease in genetic manipulation for desired characteristics, production in bulk quantity and use of cheaper growth culture media (Dey et al., 2014; Lee et al., 2015; Priji et al., 2015; Ullah et al., 2015).

Ease of genetic and environmental manipulation is very beneficial for the production of microbial lipases in a way that this allows us to produce altered enzyme with a variety of catalytic activities. Bacterial lipases may be intracellular, extracellular or attached to membrane. Extensive work has been done on various aspects of lipase production from various sources and their applications. Multiple studies on physico-chemical and catalytic properties have also been shared. However, a comprehensive review on multiple aspects of lipases is direly needed to sum up the developments happen in the field so far. Current review provides recent and detailed information about production, purification, characterization, phylogenetic analysis, engineering and applications of bacterial lipases.

## 2. Lipase producer bacterial strains

Lipase from bacterial sources is considered more suitable to withstand the hardy industrial environment. A major part of the work on the production and characterization of lipases has been focused from bacterial sources. The bacterial strains reported in the literature for the production of lipases are summarized in Table 1.

**Table 1**
Bacterial strains used in recent studies on various aspects of lipases.

| Bacterial strain | Ref. | Bacterial strain | Reference |
|---|---|---|---|
| *Acinetobacter* EH28 | (Ahmed et al., 2010) | *Geobacillus thermocatenulatus* | (Kapoor and Gupta, 2012) |
| *Acinetobacter* XMZ-26 | (Zheng et al., 2011) | *Geobacillus thermodenitrificans* | (Christopher et al., 2015) |
| *Acinetobacter baylyi* | (Uttatree et al., 2010) | *Geobacillus thermoleovorans* | (Abol-Fotouh et al., 2016) |
| *Acinetobacter radioresistens* | (Cherif et al., 2011) | *Geobacillus zalihae* | (Lee et al., 2015) |
| *Aeribacillus* 096201 | (Lokre and Kadam, 2015) | *Janibacter* sp. | (Castilla et al., 2017) |
| *Aneurinibacillus migulanus* | (Mandepudi et al., 2013) | *Lactococcus chungangensis* | (Konkit et al., 2016) |
| *Aneurinibacillus thermoaerophilus* | (Masomian et al., 2016) | *Lysinibacillus mangiferihumi* | (Tambekar and Dhandale, 2012) |
| *Bacillus amyloliquefaciens* | (Saengsanga et al., 2016) | *Microbacterium luteolum* | (Tripathi et al., 2014) |
| *Bacillus aerius* | (Saun et al., 2014) | *Micrococcus luteus* | (Akbar et al., 2014) |
| *Bacillus stearothermophilus*, *Bacillus atrophaeus* and *Bacillus licheniformis* | (Ashfaq, 2015) | *Pelosinus fermentans* | (Biundo et al., 2016) |
| *Bacillus thermocatenulatus* | (Khoramnia et al., 2011) | *Pseudoalteromonas* NJ 70 | (Wang et al., 2012) |
| *Bacillus* DH4 | (Bora and Kalita, 2008) | *Pseudomnas aeruginosa* | (Mobarak-Qamsari et al., 2011) |
| *Bacillus cereus* | (Akanbi et al., 2010; Ananthi et al., 2014) | *Pseudomonas* SPSU B3 | (Mandepudi et al., 2013) |
| *Bacillus* DOD9 | (Mahale et al., 2015) | *Pseudomonas* ADT3 | (Dey et al., 2014) |
| *Bacillus* J33 and *Bacillus* A30-1 | (Tripathi et al., 2014) | *Pseudomonas aeruginosa* | (Bisht et al., 2012) |
| *Bacillus pumilus* | (Faouzi et al., 2015a) | *Pseudomonas putida* | (Ananthi et al., 2014) |
| *Bacillus smithii* | (Chandrasekaran, 2013) | *Pseudomonas* BUP6 | (Priji et al., 2015) |
| *Bacillus sonorensis* | (Nerurkar et al., 2013) | *Pseudomonas cepacia* | (Zheng et al., 2012) |
| *Bacillus sphaericus* | (Joseph and Ramteke, 2013) | *Pseudomonas fluorescence* | (Kumar et al., 2012a,b) |
| *Bacillus stratosphericus* | (Gricajeva et al., 2016) | *Pseudomonas gessardii* | (Ramani et al., 2010; Veerapagu et al., 2013) |
| *Bacillus thermoleovorans* | (Tripathi et al., 2014) | *Pseudomonas luteola* | (Ribeiro et al., 2011) |
| *Burkholderia cepacia* | (Liu et al., 2011) | *Pseudomonas pseudoalcaligenes* | (Khoramnia et al., 2011) |
| *Burkholderia multivorans* | (Treichel et al., 2010) | *Pseudomonas stutzeri* | (Thakur et al., 2014) |
| *Burkholderia pseudomallei* | (Ooi et al., 2011) | *Psychrobacter cryohalolentis* | (Novototskaya-Vlasova et al., 2013) |
| *Burkholderia ubonensis* | (Yang et al., 2016) | *Ralstonia* | (Yoo et al., 2011) |
| *Chromobacterium viscosum* | (Bajaj et al., 2010) | *Staphylococcus* | (Kumar and Singh, 2012) |
| *Colwellia psychrerythraea* | (Do et al., 2013; Maiangwa et al., 2015) | *Staphylococcus* Lp12 | (Pogaku et al., 2010) |
| *Desulfotalea psychrophila* | (Maiangwa et al., 2015) | *Staphylococcus* sp. | (Daoud et al., 2013) |
| *Enterobacter* Bn12 | (Farrokh et al., 2014) | *Staphylococcus warneri* | (Yele and Desai, 2015) |
| *Enterococcus durans* | (Ramakrishnan et al., 2012; Salihu and Alam, 2015) | *Stenotrophomonas maltophilia* | (Li et al., 2016a) |
| *Enterococcus faecium* | (Ramakrishnan et al., 2016) | *Streptomyces lividans* | (Wang et al., 2016) |
| *Geobacillus* EPT9 | (Zhu et al., 2015) | *Thalassospira permensis* | (Kai and Peisheng, 2016) |
| *Geobacillus stearothermophilus* | (Dror et al., 2015) | *Xanthomonas oryzae* | (Mo et al., 2016) |
| *Geobacillus stearothermophilus* | (Ekinci et al., 2015) | *Yersinia enterocolitica* | (Ji et al., 2015) |

## 3. Phylogeny and genetic basis of lipase producing bacteria

Comparison among the sequences of different bacterial lipases highlighted the fact that they appear to be quite variable. Lipases carry a wide diversity of properties both on biochemical or molecular level. The only known conserved sequence common among all lipases is Gly-X-Ser-X-Gly pentapeptide, which encloses the active site serine residue. For molecular phylogenetic analysis of different lipase producing bacteria (Table 1), sequences of 42 proteins representing 7 diverse phylogenetic clusters are used here (Fig. 1). Further sub-clusters were observed in each group on the basis of closeness in the sequences of lipase proteins. The sub-clusters showed further branching based on the closeness of lipase protein sequences and revealed significant diversity among lipase producing bacteria. Analysis of residues around GxSxG motif also demonstrated existence of diversity among different lipase producing bacteria and provided an insight to the closeness of lipases of different bacteria based on amino acid sequences. Such information could be utilized for the designing of probe or primers for studying lipase genes of diverse groups and classification of newly identified lipases (da Silva et al., 2013; Leathers et al., 2013; Masomian et al., 2016).

These lipases are classified on the basis of their biochemical properties. However, in current era of genomics and bioinformatics, significant number of new lipases have been discovered that deviate from existing criteria of classification (Arpigny and Jaeger, 1999; Eggert et al., 2001; Jaeger et al., 1994). Moreover, the ever increasing information about protein structure by using techniques like X-ray crystallography or NMR spectroscopy, has added considerable uncertainty about the usefulness of current classification system (Castilla et al., 2017; Masomian et al., 2016). Therefore, a comprehensive attempt is required in this regard to provide a more representative criteria for the classification of lipases.

## 4. Purification of bacterial lipases

The purpose of purification is not only to isolate the enzymes from contaminants but also to improve their activity, stability and shelf life. Structural and conformational studies can be conducted after the proteins are purified up to homogeneity level (Nadeem et al., 2009, 2015a). Kinetic and thermodynamic mechanism of lipases for substrate hydrolysis, transesterification reaction and structure-function relationship can only be established for purified lipases. Moreover, purified lipases are needed to make important formulations for industrial and medicinal uses. Purification is a key step that is performed to interpret the actual function of a specific

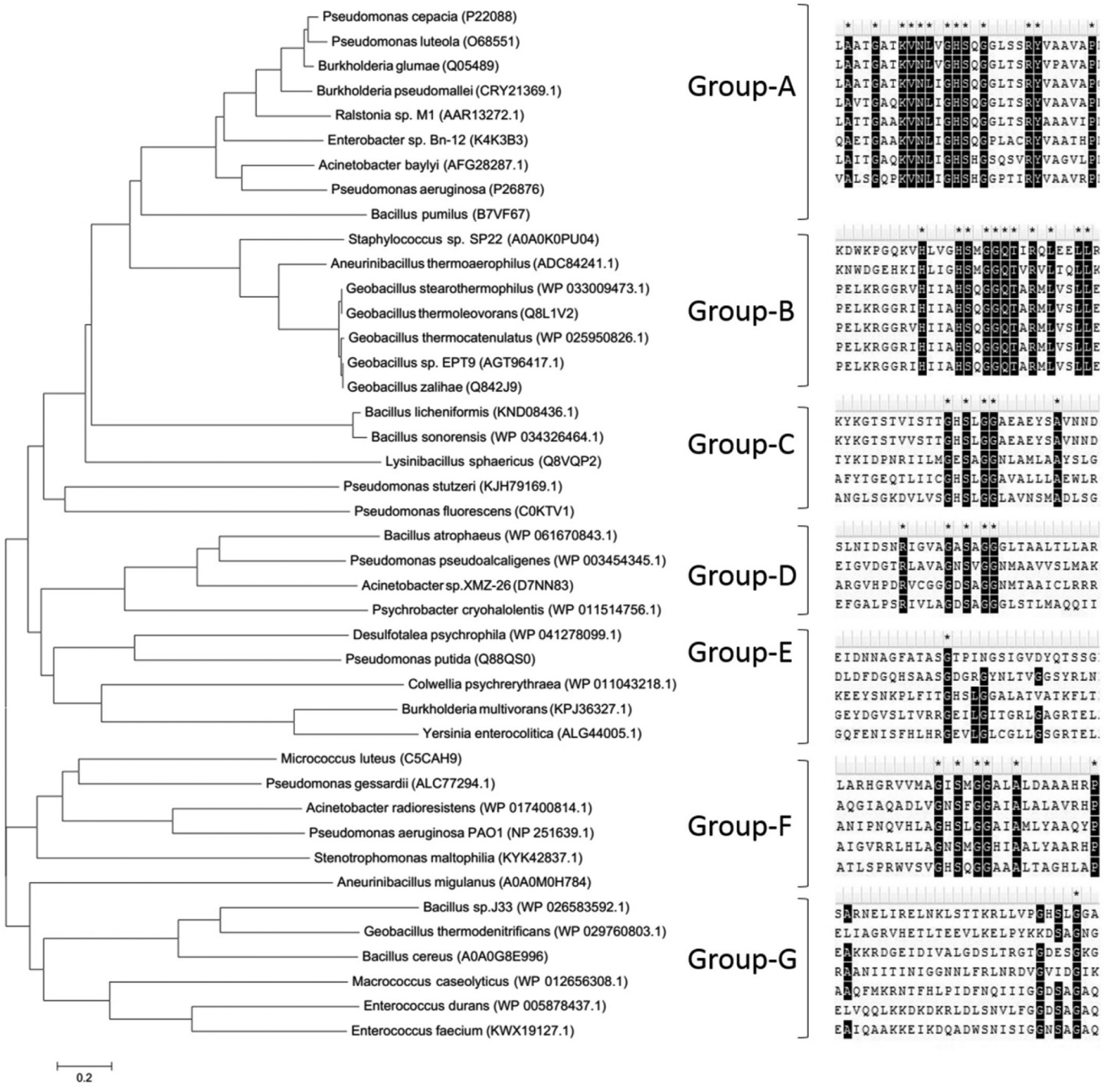


**Fig. 1.** Phylogenetic analysis and multiple sequence alignment showing the relationship among different lipase producing bacteria. Rooted phylogenetic tree was built by using the Neighbor-Joining method [75]. The bootstrap consensus tree was inferred from 1000 replicates. The optimal tree with the sum of branch length = 27.62428903 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [76] and were in the units of the number of amino acid substitutions per site. The analysis involved 42 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 118 positions in the final dataset. The length of the branches is proportional to the relative phylogenetic distance between the proteins. Evolutionary analyses were conducted in MEGA6 [77]. Multiple sequence alignment was performed by using Cluastal W. for alignment of 29 amino acid sequences surrounding GxSxG motif.

enzyme. Bacterial cells are removed from culture broth after the fermentation process to get extracellular lipases. The cell free extract is then concentrated by extraction with organic solvents, ultrafiltration or by ammonium sulphate precipitation. Ammonium sulphate precipitation is mostly used during early stages of purification, considered as a crude separation step and is followed by a combination of chromatographic steps (Saxena et al., 2003). Broth or cell culture is passed through various stages starting from salt precipitation to column chromatography depending upon the nature of the proteins and desired level of purification. Various chromatographic techniques including anion exchange, cation exchange and size exclusion chromatography are used for the purification of lipases. Data from various studies suggested that lipases had been purified from 2.4 to 500 fold purification with 10.3–36% overall yield (Table 2).

**Table 2**
Purification strategies recently used for various studies of bacterial lipases.

| Strain | Purification steps | Purification (fold)/ Yield (%) | Reference |
|---|---|---|---|
| *Burkholderia ubonensis* SL-4 | Ammonium sulphate precipitation, Q sepharose FF anion exchange and superdex 75 gel filtration chromatography. | 68.5/13.34 | (Yang et al., 2016) |
| *Bacillus* sp. | Ammonium sulphate precipitation andion-exchange chromatography | 5.1/10.5 | (Sivaramakrishnan and Incharoensakdi, 2016) |
| *Pseudomonas aeruginosa* BUP2 | Ammonium sulphate precipitation and sephadex G-100 gel filtration | 36/20 | (Unni et al., 2016) |
| *Xanthomonas oryzae* pv. *oryzae* YB103 | Ammonium sulphate precipitation and phenyl sepharose chromatography | 101.1/15.7 | (Mo et al., 2016) |
| *Bacillus pumilus* | Ammonium sulphate precipitation, heat treatment (30 min at 70 °C), sephacryl S-200 and Mono-S chromatography | 210/36 | (Faouzi et al., 2015a) |
| *Geobacillusstearothermophilus* AH22 | Heat treatment (30 min at 70 °C), DEAE-cellulose, sephadex G-150, sephadex G-25 | 18.3/19.7 | (Ekinci et al., 2015) |
| *Yersinia enterocolitica* KM1 | Ultrafiltration, ammonium sulphate precipitation, sephacry™ HRS-100, superdex G-75 | 26.0/10.3 | (Ji et al., 2015) |
| *Idiomarina* sp. W33 | Ammonium sulphate precipitation, DEAE-sepharose, sephacryl S-200 | 7.4/24.6 | (Li et al., 2014) |
| *Pseudomonas aeruginosa* AAU2 | Ammonium sulphate precipitation, gel permeation chromatography | 4.96/54.1 | (Bose and Keharia, 2013) |
| *Staphylococcus* sp. | Ammonium sulphate precipitation, S-200 | 23.92/32 | (Daoud et al., 2013) |
| *Aneurinibacillus thermoaerophilus* HZ | Q-Sephaorse anion exchange chromatography and sephadex G-75 gel filtration | 15.6/19.7 | (Masomian et al., 2013) |
| *Stenotrophomonas maltophilia* CGMCC 4254 | Ammonium sulphate precipitation, HIC | 60.54/8.4 | (Li et al., 2013) |
| *Bacillus pumilus* RK31 | Speed centrifuge, ammonium sulphate precipitation, gel filtration sephadex G-200, DEAE cellulose (Ion exchange) | 186/NR | (Kumar et al., 2012a,b) |
| *Pseudoalteromonas* sp. NJ 70 | Ammonium sulphate precipitation, phenyl-sepharose 6FF, DEAE sephadex A-50 | 27.5/25.4 | (Wang et al., 2012) |
| *Chromohalobacter* sp. LY7-8 | Ammonium sulphate precipitation, sephacryl S-100 | 10.2/12.9 | (Xin and Hui-Ying, 2012) |
| *Halobacillus* sp. strain LY5 | Ammonium sulphate precipitation, DEAE cellulose, sephacryl S-100 | 8.7/10.3 | (Xin et al., 2012) |
| *Staphylococcus aureus* | Phenyl sepharose CL-4B chromatography, superose-12 chromatography | 6.76/20 | (Sarkar et al., 2012) |
| *Bacillus subtilis* NS 8 | Ultrafiltration, DEAE-Toyopearl, sephadex G-75 | 500/16 | (Olusesan et al., 2011) |
| *Amycolatopsis mediterranei* DSM 43304 | Ammonium sulphate precipitation, Q sepharose HP and toyopearl phenyl-650 column chromatography | 398/36 | (Dheeman et al., 2011) |
| *Ralstonia* sp. CS274 | Ammonium sulphate precipitation, ultrafiltration and phenyl sepharose CL-4B column chromatography | 4/20.8 | (Yoo et al., 2011) |
| *Geobacillus* sp. ARM (expressed in *E. coli* TOP10) | Immobilized metal affinity chromatography (IMAC) | 14.6/63.2 | (Ebrahimpour et al., 2011) |
| *Staphylococcus warneri* EX17 | Octyl-sepharose, butyl-toyopearl | 18.7/70 | (Volpato et al., 2011) |
| *Acinetobacter* sp. XMZ-26 | Affinity chromatography | 2.4/65.77 | (Zheng et al., 2011) |
| *Acinetobacter* sp. EH28 | Ammonium sulphate precipitation, ultrafiltration and phenyl-sepharose hydrophobic interaction chromatography | 24.2/47 | (Ahmed et al., 2010) |
| *Pseudomonas aeruginosa* BN-1 | Ultrafiltration, sephadex G-100 and DEAE Sephadex A-50 column chromatography | 43/NR | (Syed et al., 2010) |
| *Spirulina platensis (Arthrospira)* | Ammonium sulphate precipitation, DEAE sepharose, sepharose-6B | 375/29.35 | (Demir and Tükel, 2010) |
| *Pseudomonas aeruginosa* LX1 | Ammonium sulphate precipitation, DEAE sepharose FF | 4.3/41.1 | (Ji et al., 2010) |
| *Pseudomonas aeruginosa* CS-2 | Ultra filtration, acetone precipitation, DEAE sephadex A-50 | 25.5/45.5 | (Peng et al., 2010) |
| *Pseudomonas gessardii* | Ammonium sulphate precipitation, DEAE cellulose, sephadex G-25 | 7.59/16.2 | (Ramani et al., 2010) |
| *Acinetobacter baylyi* | Ammonium sulphate precipitation, sephadex G-75 | 21.9/13.5 | (Uttatree et al., 2010) |

NR = Not Reported.

## 5. Characterization of bacterial lipases

### 5.1. Physiochemical properties of bacterial lipases

Physiochemical properties of twenty seven recently studied (2010–2017) bacterial lipases are summarized in Table 3. It can be concluded from these studies that all bacterial lipases except that of *Halobacillus* sp. strain LY5 (Xin et al., 2012) and *Pseudomonas gessardii* (Ramani et al., 2010) have molar masses less than 70 kDa. A minimum molar mass of 19 and 19.2 kDa was observed for lipases from *Bacillus stratosphericus* and *Enterococcus faecium* by Gricajeva et al. (2016) and Ramakrishnan et al. (2016), respectively. Lipases obtained from *Pseudomonas gessardii*, *Spirulina platensis* and *Bacillus pumilus* RK31worked optimally at acidic pH, while all other bacterial lipases worked at alkaline pH. *Cohnella* sp. A01 lipase studied by Golaki et al. (2015) showed maximum activity at 70 °C, while all other bacterial lipases showed optimal activity below 70 °C. Zheng et al. (2011) reported a minimum (15 °C) optimum temperature for cold active *Acinetobacter* sp. XMZ-26 lipase.

### 5.2. Kinetic properties of bacterial lipases for substrate hydrolysis

A variety of para-Nitrophenyl (p-NP) esters having $C_2$ to $C_{16}$ (p-NP acetate to p-NP palmitate) in their fatty acid side chain can be hydrolyzed by bacterial lipases. Kinetics of substrate hydrolysis for different esters are summarized in Table 4. The affinity of a lipase for substrate hydrolysis is determined by Michaelis constant ($K_m$) that is the substrate concentration at which the rate of reaction is half of the maximum rate ($V_{max}$). $V_{max}$ is the maximum rate when an enzyme is fully saturated with substrate concentration (Nadeem et al., 2015b). *Streptomyces lividans* lipase reported by Wang et al. (2016) have high affinity ($K_m$ = 0.0064 mM) for the substrate (p-NP caproate) but have the lowest value of catalytic constant ($K_{cat}$ = 0.1665 $s^{-1}$) and specificity constant ($K_{cat}/K_m$ = 26.02 $s^{-1}$/mM). However, *Acinetobacter* sp. XMZ-26 lipase studied by Zheng et al. (2011) have high affinity ($K_m$ = 0.075 mM) for the substrate (p-NP octanoate) as well as high catalytic constant ($K_{cat}$ = 560.52 $s^{-1}$) and highest specificity constant ($K_{cat}/K_m$ = 7377 $s^{-1}$/mM). Catalytic properties of the enzymes are dependent on various

**Table 3**
Physiochemical properties of recently studied bacterial lipases.

| Strain | Mass. (kDa) | pH Opt. | Temp. Opt. (°C) | Ref. |
|---|---|---|---|---|
| *Burkholderia ubonensis* | 33 | 8.5 | 65 | (Yang et al., 2016) |
| *Enterococcus faecium* | 19.2 | 10.8 | 40 | (Ramakrishnan et al., 2016) |
| *Pseudomonas aeruginosa* | 29 | 8.0 | 45 | (Unni et al., 2016) |
| *Bacillus stratosphericus* | 19 | 9.0 | 35 | (Gricajeva et al., 2016) |
| *Streptomyces lividans* | 31.43 | 8.0 | 50 | (Wang et al., 2016) |
| *Bacillus* sp. | 24 | 6.5 | 37 | (Sivaramakrishnan and Incharoensakdi, 2016) |
| *Bacillus amyloliquefaciens* E1PA | 23 | 10 | 40 | (Saengsanga et al., 2016) |
| *Geobacillus* sp. EPT9 | 44.8 | 8.5 | 55 | (Zhu et al., 2015) |
| *Cohnella* sp. A01 | 29.5 | 8.5 | 70 | (Golaki et al., 2015) |
| *Bacillus pumilus* | 27 | 8.0 | 45 | (Faouzi et al., 2015a) |
| *Geobacillus thermodenitrificans* | 50 | 9.0 | 65 | (Christopher et al., 2015) |
| *Yersinia enterocolitica* | 34.3 | 9.0 | 37 | (Ji et al., 2015) |
| *Pseudomonas aeruginosa* | 40 | 9–10 | 40 | (Sarac et al., 2015) |
| *Microbacterium* sp. | 40 | 8.5 | 50 | (Tripathi et al., 2014) |
| *Enterobacter* sp. Bn12 | 31.3 | 8.0 | 60 | (Farrokh et al., 2014) |
| *Idiomarina* sp. W33 | 67 | 7–9 | 60 | (Li et al., 2014) |
| *Staphylococcus* sp. | 38 | 8.0 | 45 | (Daoud et al., 2013) |
| *Colwellia psychrerythraea* 34H | 34.5 | 7.0 | 25 | (Do et al., 2013) |
| *Chromohalobacter* sp. LY7-8 | 44 | 9.0 | 60 | (Xin and Hui-Ying, 2012) |
| *Bacillus pumilus* RK31 | 62.2 | 6.0 | 60 | (Kumar et al., 2012a,b) |
| *Pseudoalteromonas* sp. NJ 70 | 37 | 7.0 | 35 | (Wang et al., 2012) |
| *Halobacillus* sp. strain LY5 | 96 | 10 | 50 | (Xin et al., 2012) |
| *Ralstonia* sp. | 28 | 8–9.5 | 45 | (Yoo et al., 2011) |
| *Acinetobacter* sp. XMZ-26 | 35.5 | 10 | 15 | (Zheng et al., 2011) |
| *Acinetobacter baylyi* | 30 | 8.0 | 60 | (Uttatree et al., 2010) |
| *Spirulina platensis* | 45 | 6.5 | 45 | (Demir and Tükel, 2010) |
| *Pseudomonas gessardii* | 92 | 5.0 | 30 | (Ramani et al., 2010) |
| *Janibacter* sp. R02 | 44 | 8–9 | 80 | (Castilla et al., 2017) |

**Table 4**
Kinetic properties of bacterial lipases for substrate hydrolysis.

| Strain | Substrate | $V_{max}$ ($\mu$M min$^{-1}$) | $K_m$ (mM) | Specific activity (U/mg) | $K_{cat}$ ($s^{-1}$) | $K_{cat}/K_m$ ($s^{-1}$/mM) | Reference |
|---|---|---|---|---|---|---|---|
| *Burkholderia ubonensis* | p-NP acetate | NR | 3.89 | 73.14 | 166.6 | 42.82 | (Yang et al., 2016) |
| | p-NP butyrate | NR | 3.77 | 167.06 | 287.6 | 76.29 | |
| | p-NP caprylate | NR | 2.94 | 203.53 | 236 | 80.27 | |
| | p-NP decanoate | NR | 1.30 | 241.03 | 221 | 169.91 | |
| | p-NP laurate | NR | 0.77 | 303.65 | 259.3 | 336.69 | |
| | p-NP myristate | NR | 0.72 | 362.82 | 391.6 | 543.93 | |
| | p-NP palmitate | NR | 2.36 | 86.22 | 351.7 | 149.03 | |
| *Pseudomonas aeruginosa* | p-NP palmitate | 999 | 4.75 | 2392 | NR | NR | (Unni et al., 2016) |
| *Bacillus stratosphericus* | p-NP butyrate | 1100 | 0.05 | NR | 76.7 | 1533.4 | (Gricajeva et al., 2016) |
| | p-NP caprylate | 2500 | 0.034 | NR | 165 | 4862.9 | |
| | p-NP decanoate | 100 | 1.94 | NR | 83.33 | 44.5 | |
| *Streptomyces lividans* | p-NP acetate | 48.40 | 0.535 | 7.65 | 38.9 | 72.86 | (Wang et al., 2016) |
| | p-NP butyrate | 10.51 | 0.143 | 4.66 | 8.45 | 59.29 | |
| | p-NP caproate | 0.21 | 0.0064 | 1.13 | 0.167 | 26.02 | |
| *Geobacillus thermodenitrificans* | p-NP laurate | 0.556 | 0.44 | NR | NR | NR | (Christopher et al., 2015) |
| *Yersinia enterocolitica* | p-NP butyrate | $5.24 \times 10^5$ | 16.58 | NR | $10^4$ | 621 | (Ji et al., 2015) |
| *Ralstonia* sp. | p-NP palmitate | $1.01 \times 10^5$ | 2.73 | NR | NR | NR | (Yoo et al., 2011) |
| *Acinetobacter* sp. XMZ-26 | p-NP acetate | NR | 1.563 | NR | 2565 | 1641 | (Zheng et al., 2011) |
| | p-NP butyrate | NR | 0.867 | NR | 2452 | 2829 | |
| | p-NP octanoate | NR | 0.075 | NR | 560.5 | 7377 | |
| | p-NP decanoate | NR | 0.155 | NR | 589.7 | 3809 | |
| | p-NP myristate | NR | 0.282 | NR | 58.86 | 208.5 | |
| | p-NP palmitate | NR | 0.349 | NR | 22.18 | 63.57 | |
| *Spirulina platensis* | p-NP palmitate | 38.9 | 0.02 | NR | 30 | 1500 | (Demir and Tükel, 2010) |

p–NP = para–Nitrophenyl, NR = Not Reported.

factors including type and concentration of the substrate, concentration of enzyme, pH and temperature.

## 6. Engineering/modification of bacterial lipases

Bacterial lipases are considered very important for a number of applications and it has accelerated the search for new lipases and variants from natural sources (Li et al., 2016a). In most of the cases, the properties of native enzymes need to be optimized for industrial applications. There are a number of methods being employed to introduce desired characteristics in lipases, which include chemical modification, immobilization, UV and gamma rays irradiations, amino acid tailoring and site directed mutagenesis. At present, genetic engineering is considered as the most convenient and accurate method to make a “tailor-made” enzyme.

Protein engineering involves two major strategies: rational design and directed evolution (Bornscheuer, 2013, 2008). Rational design requires two types of information about proteins which

include data of three-dimensional structure and relation between protein structure and function (Lan et al., 2015). Contrary to rational design, such information is not required for directed evolution studies (Porter et al., 2016), which involves random mutagenesis of the target gene, generation of mutant library and screening to recognize a variant having desired characteristics (Bornscheuer, 2008). The generation of mutant library involves two approaches: a non-recombining and a recombining evolution. In the first approach, point mutations are introduced in a target gene. For this purpose different techniques including UV irradiation, chemical mutagenesis or error-prone polymerase chain reaction (epPCR) are used. The recombining evolution is based on recombination principle. As a first step, a pool of recombinant chimera is generated by shuffling and reconstruction of several parental genes. The sources of parental genes may comprise either mutants of a single gene (generated by other mutagenesis methods) or naturally occurring homologs of a gene family (Bornscheuer, 2008; Lülsdorf et al., 2015; Porter et al., 2016). The recombinant variants of gene are cloned followed by transformation and expression in a host system. At the end, a high-throughput screening is executed to isolate a variant with the best desired characteristics. Both the recombining and non-recombining evolution methods are performed *in vivo* (directly on colonies growing on agar plates) or *in vitro* (on cultures of individual clones) (Hasan et al., 2009).

Molecular engineering technologies have been applied to better understand the catalytic mechanism or have been used to improve the specific properties of microbial lipases (Table 5). Recently, protein engineering for thermo-stable (Ahmad and Rao, 2009; Gumulya and Reetz, 2011; Khurana et al., 2011; Shih and Pan, 2011; Wu et al., 2009) and organic solvent-tolerant lipases (Dror et al., 2014; Kawata and Ogino, 2010; Monsef-Shokri et al., 2014; Reetz et al., 2010; Yedavalli and Rao, 2013) has been the focus of research. Improvement of properties like inherent stability against high temperatures and capacity to tolerate harsh organic solvents have substantial future in an array of synthetic reactions in industry (Masomian et al., 2010). Similarly, the bio-catalytic properties of lipases serve as an important environment friendly substitute to the conventional chemical approaches. Though, lipase-catalyzed processes hold eminent commercial worth, yet the use of lipases is restricted owing to low yields, stumpy reproducibility, and inconsistent optimal performance in native form. Protein engineering by recombinant DNA technology is providing appropriate means to overcome these shortcomings and to produce robust enzyme catalysts at high yields (Ema et al., 2012; Knapp et al., 2016; Mohammadi et al., 2016; Wi et al., 2014). With the increasing understanding of the mechanisms for the regulation of gene expression, different expression systems have currently been optimized for improved functional characteristics and new hyper-producing strains (Knapp et al., 2016). An increased yield of recombinant lipases is important on industrial scale because it is required for increased volumetric productivity, reduced downstream purification costs, and much purer resulting crude enzyme.

## 7. Applications of bacterial lipases

Ease of availability (due to production at industrial level), ability to work in heterogeneous media, interfacial activation phenomenon and large scale substrate specificity are the factors due to which lipases are largely used in various industrial applications (Hasan et al., 2006; Kapoor and Gupta, 2012). Moreover, being non-toxic and eco-friendly, lipase are considered more suitable as compared to other chemical or synthetic catalysts. Therefore, widely used in food, dairy, flavor, detergent, pharmaceuticals, biofuels and cosmetics industries. Esters having natural flavor are formed by esterification and trans-esterification using lipases (Rajendran et al., 2009). Detergent industries consume almost 1000 tons of lipases every year in hydrolysis reaction to remove oil stains (Hasan et al., 2006; Parra et al., 2015).

### 7.1. Food industry

Lipases are used for the hydrolysis of milk fat, cheese ripening, flavor enrichment and lipolysis of butter fat in dairy industry (Adrio and Demain, 2014; Boonmahome and Mongkolthanaruk, 2013; Farrokh et al., 2014; Ferreira-Dias et al., 2013; Konkit and Kim, 2016; Rigo et al., 2010; Sirisha et al., 2010; Ullah et al., 2015). Halotolerant lipase obtained from *Lactobacillus plantarum* is used in the synthesis of different fermented food products such as sour dough, olives, vegetable sausages and cheese (Esteban-Torres et al., 2015). *Pseudomonas* lipase was considered to be useful in food processing and oil manufacturing. Castor oil is a unique vegetable oil that contains high amounts (90%) of a hydroxy monounsaturated fatty acid named ricinoleic acid. This industrially important acid can be obtained by hydrolysis of castor oil using more efficient lipase-catalyzed process (Goswami et al., 2012). Cocoa butter equivalents are produced by lipase catalyzed inter-esterification of natural triglycerides, such as middle fraction of palm oil or sunflower. Moreover, lipase can be used to enhance the shelf life, flavors and rheological properties of fruit juices, soups, sauces, cheese and baked foods. The shelf life of different bakery products and their

**Table 5**
Improved properties of bacterial lipases through engineering.

| Improved property | Name of Lipase | Bacteria | Method of engineering | Reference |
|---|---|---|---|---|
| Surface hydrophobicity | BTL2 | *Bacillus thermocatenulatus* | Site-directed mutagenesis of lid domain | (Tang et al., 2015) |
| Protein activity | Lipase PS | *Burkholderia cepacia* | Rational design | (Ema et al., 2012) |
| | Lipase BpL5 | *Bacillus pumilus* | Point mutation | (Wi et al., 2014) |
| | lipase A | *Serratia marcescens* | Rational design | (Mohammadi et al., 2016) |
| Thermostability | lip | *Bacillus* sp. | epPCR | (Khurana et al., 2011) |
| | lipase A | *Bacillus subtilis* | Iterative saturation mutagenesis | (Gumulya and Reetz, 2011) |
| | lipase A | *Bacillus subtilis* | Site-saturation mutagenesis | (Ahmad and Rao, 2009) |
| | r03Lip | *Geobacillus* sp. | epPCR and site saturation mutagenesis | (Shih and Pan, 2011) |
| | lipGRD | *Geobacillus* sp. | Rational design | (Wu et al., 2009) |
| Stability in organic solvents | C7E3F2 lipase | *Pseudomonas* sp. | Site-directed mutagenesis | (Monsef-Shokri et al., 2014) |
| | LipA | *Bacillus subtilis* | Site saturation mutagenesis | (Yedavalli and Rao, 2013) |
| | lipase T6 | *Geobacillus stearothermophilus* | Random mutagenesis, Structure-guidedconsensus | (Dror et al., 2014) |
| | LipA | *Bacillus subtilis* | Iterative-saturation mutagenesis | (Reetz et al., 2010) |
| | LST-03 lipase | *Pseudomonas aeruginosa* | Rational design | (Kawata and Ogino, 2010) |
| Proteolytic resistance | LipA | *Bacillus subtilis* | Loop scanning, site-saturation mutagenesis | (Ahmad et al., 2012) |
| Substrate tolerance | TTL | *Thermoanaerobacter thermohydrosulfuricus* | Genetic code engineering | (Hoesl et al., 2011) |
| Increased production | lipAB | *Burkholderia glumae* | Random mutagenesis | (Knapp et al., 2016) |

softness can be improved with lipases. Softness of noodles can also be enhanced with the help of lipases. Phospholipases also found to be used in the treatment of egg yolk for the manufacturing of mayonnaise and various emulsifiers (Ray, 2012). *Acinetobacter* sp. EH28 lipase is used to make flavor esters like ethyl butyrate, ethyl valerate and ethyl caprylate in organic solvents. Ethyl caprylate, which has a fruity-flowery fragrance, is applied to give different fruity flavors like peach, apple, banana and pineapple (Ahmed et al., 2010). Lipases are also applied for flavor modification and gelling in fish flesh for protein polymerization (Joseph et al., 2007). One important application of lipase is in hydrolysis of vegetable oil for the generation of free acids which are further used in food, soap and biomedical industries.

*Staphylococcus* lipases are used as starter culture in fermentation of sausages to develop typical flavor. Various esters such as monoglycerides, isoamyl aceteate (banana flavor), valerate and hexyl acetate (pear flavor) and butyl acetate (pineapple flavor) are synthesized by using immobilized *Staphylococcus* lipase. Monoglycerides are nonionic surfactants have hydrophilic as well as hydrophobic regions and are used in food emulsifiers for bakery products, margarines, dairy products and sauces. Less value lipids can be modified to high value products by the action of lipase enzyme. These enzymes changed the location of fatty acid's chain in the glycerides and replace with a new one. Lipases from *Pseudomonas, Alcaligenes* and *Achromobacter* are known to withstand the pasteurization process and affect flavor development during cheese ripening. Lipolytic lactic acid bacteria are also involved in vegetable fermentations and ripening of some Italian sausages (Jaeger et al., 1994). Use of phospholipases to remove phospholipids from vegetables (de-gumming) is an environment friendly process (Horchani et al., 2012).

### 7.2. Pharmaceuticals

A thermo-stable lipase obtained from *Acinetobacter baylyi* also resistant to organic solvents works as an efficient catalyst in bio-energy, pharmaceutical industries and for trans-esterification of palm oil to FAMEs (Uttatree et al., 2010). Moreover, lipases also have medicinal uses such as skin scalp disease and hair loss can be treated by using this enzyme (Sangeetha, 2011). Cold active lipases are used to make different useful chemical compounds such as aryl aliphatic glycolipids, citronellol laurate from citronellol and lauric acid, and ethyl esterification of docosahexaenoic acid to ethyl docosahexaenoate. *Bacillus* lipases showed selectivity to the fatty acid chain length of an ester, and few enzymes display positional specificity. Due to these properties, *Bacillus* lipases can be used in pharmaceutical industries for the synthesis of enantiopure compounds (Guncheva and Zhiryakova, 2011). Antioxidants such as tyrosol acetate, propyl gallate and eugenol benzoate are formed by using *Staphylococcus* lipase (Horchani et al., 2012).

Lipase can be used for the diagnostic purposes such as in case of tuberculosis (TB). Lipase secreted by *Mycobacterium tuberculosis* can be detected to check the infection with high specificity and sensitivity. Level of lipase in blood serum can be used as diagnostic tool for the detection of acute pancreatitis and pancreatic injury. Acute pancreatitis is usually caused due to misuse of alcohol or bile duct obstruction. Lipases are used in making hair waving, as a component of topical anti-obese creams. These are also used as digestive aids and for the treatment of malignant tumors because lipases are found as activators of tumor necrosis factor (Nagarajan, 2012).

### 7.3. Biofuels

Trans-esterification process catalyzed by lipase is performed in the presence of fatty acids and short chain alcohol usually methanol (Lotti et al., 2015). Methanol and waste cooking oil are converted into biodiesel and glycerol in the presence of lipases (Karmee et al., 2015). *Acinetobacter venetianus* RAG-1 lipase is used for the production of biodiesel by trans-esterification (Boonmahome and Mongkolthanaruk, 2013). Micro crystals of $K_2SO_4$ coated with lipase are used as an alternative to chemical catalysts for the production of biodiesel (Sirisha et al., 2010; Zheng et al., 2012). Alkali stable lipases are more useful in the production of biodiesel because alkaline pH is useful to improve oil solubility and homogeneity of the reaction mixture giving high trans-esterification rates and biodiesel production (Christopher et al., 2015; Li et al., 2016b). Lipase trans-esterification reduced the downstream processing cost in biodiesel production (Hegde et al., 2015). *Burkholderia cepacia* lipase immobilized on NKA resin is used to make a high value biocatalyst and for the production of biodiesel (Liu et al., 2011). In fact, cross linking of *Burkholderia cepacia* lipase with glutaraldehyde and then entrapment in a hybrid matrix of equal proportions of alginate and k-carrageenan used for biodiesel production. This immobilized lipase remained active after six cycles of reuse. So this is a good source for biodiesel production industry (Abdulla and Ravindra, 2013). Lipase from *Ralstonia* sp. catalyzed the production of biodiesel in the presence of soya bean and palm oil but more efficiently in the latter case at pH 8 with 5% methanol and 20% water content (Yoo et al., 2011). Moreover, lipases are used as biocatalysts to make biodegradable polymers such as 1-butyl oleate, which is used in biodiesel to reduce the viscosity in winter season. Tri-methylolpropane esters originated from lipases are used as lubricants. Lipase isolated from *Bacillus* sp. showed high activity towards oleic rich oils. Lipase immobilized on celite could retain 90% lipase activity after eight cycles. Trans-esterification of oil using the immobilized lipase obtained from *Botryococcus* sp. resulted in 80% yield of fatty acid methyl esters which had good properties for use as biodiesel (Sivaramakrishnan and Incharoensakdi, 2016).

A new lipase SL-4 from *Burkholderia ubonensis* was employed to catalyze soybean oil for biodiesel production, the liquid lipase SL-4 could secure a conversion ratio of 92.24% in a solvent-free system. That resulted in a new thermo-solvent-stable lipase possessing an attractive potential for biotechnological applications as biocatalyst, especially for biodiesel production (Yang et al., 2016).

### 7.4. Detergents

The chemical ingredients of detergents are hazardous to human beings and cause environmental pollution therefore, lipases are used as substitute of these harmful ingredients. Most of the companies are currently producing enzyme based detergents. Lipase based detergents digest the lipid molecules from the soiled substrates, active at the ambient temperature and preferred for long life of cleaned fabric.

Lipase obtained from *Pseudomonas* ADT3 was found useful in detergent (Dey et al., 2014). Lipase from *Bacillus sonorensis* when mixed with detergent can remove corn oil stains from un-dyed cotton fabric (Nerurkar et al., 2013). Cold active lipases are applied as additives in detergent preparations that can be used in laundry to wash clothes at low temperature (Aboualizadeh et al., 2011) and in organic synthesis of chiral intermediate (Zheng et al., 2011). The alkaline and thermotolerant lipase produced by *Pseudomonas aeruginosa* strain BUP2 have a high specific activity and is efficiently used in detergent industry (Unni et al., 2016). Lipases of *Bacillus flexus* XJU-1, *Bacillus licheniformis*, *Bacillus licheniformis* VSG1, *Bacillus pumilus* SG2, *Bacillus subtilis* JPBW-9, *Geobacillus* sp., *Pseudomonas aeruginosa* san-ai and *Serratia marcescens* DEPTK21 are frequently used in detergents (Niyonzima and More, 2015).

### 7.5. Other applications of lipases

Bacterial lipases are also applied in the formation of biopolymers. *B. multivorans* V2 was observed to produce solvent-tolerant lipase used for the synthesis of ethyl butyrate ester in non-aqueous environment (Sarethy et al., 2011). Omega- 3 polyunsaturated fatty acids are prepared by alcoholysis of cod liver oil in the presence of *Pseudomonas* lipase (Pallavi et al., 2014). Lipase obtained from *Pseudomonas* ADT3 was found useful in leather and chemical industries (Dey et al., 2014). Lipases are also used in the bioremediation of different industrial and municipal wastes (Shafqat et al., 2015).

Psychrophilic lipases have become important due to their increased use in organic synthesis of chiral intermediates because these enzymes perform at low temperature and show high activity in cold conditions, which are in turn favorable conditions for the synthesis of delicate compounds (Wiese et al., 2010). Lipase from *Pseudomonas* species (KWI-56) is used to enhance whiteness of paper and useful in paper recycling. Lipase has been applied to increase the pulping rate in paper industry and to remove various hydrophobic and lipid fractions of the wood (Hasan et al., 2006).

Ricinoleic acid is important fatty acid obtained after the lipase catalyzed hydrolysis of oil used as raw material for resins and thermosetting acrylics, as pigment and dye disperser in printing ink, textile finishing, as wetting agent, provides flexibility and softness to leather (Goswami et al., 2012). The chemical leather process can be substituted with thermoalkaliphilic lipase for boosting the quality of leather and reducing the environmental hazards (Abol-Fotouh et al., 2016). In the cosmetic industry, monoglycerides are used to improve the consistency of creams and lotions. Moreover, insecticides are also formed by resolution of racemic alcohols/esters (Kapoor and Gupta, 2012). Lipase produced from *Pseudomonas* sp. can be used to produce compounds like isopulegol which has citrus type fragrance and β-pinene which gives spearmint flavor (Gupta et al., 2015).

## 8. Conclusion

Bacterial lipases are an important group of enzymes that offer enormous potential for various applications, and there is considerable interest in identifying and developing novel bacterial lipases. Detailed study of the latest literature indicates that lipases are one of the most produced bacterial enzymes. Many researchers worldwide are conducting their studies on isolation, screening and optimizing the conditions for bacterial strains to gain maximum production of lipases. Versatile application of lipases is the justification of all these efforts. Although the cold active and thermo tolerant bacterial strains and their products (lipases) have been identified but there is a large vacuum for engineered lipases with significant characteristics for specific applications. The properties of lipases that need to be improved are stability and turnover under application conditions. They need to be robust and versatile with respect to the range of substrates they can act on, but at the same time they should have a high specificity for the reactions they catalyze.

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