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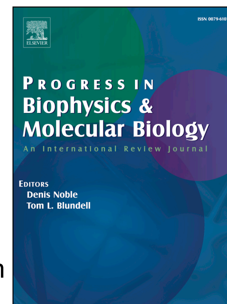

Bacterial lipases: A review on purification and characterization

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1 # Bacterial Lipases: A review on purification and
2 # characterization

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31 **ABSTRACT**

32 Lipase (E.C.3.1.1.3) belongs to the hydrolases and is also known as fat splitting, glycerol
33 ester hydrolase or triacylglycerol acylhydrolase. Lipase catalyzes the hydrolysis of triglycerides
34 converting them to glycerol and fatty acids in an oil-water interface. These are widely used in
35 food, dairy, flavor, pharmaceuticals, biofuels, leather, cosmetics, detergent, and chemical
36 industries. Lipases are of plant, animal, and microbial origin, but microbial lipases are produced
37 at industrial level and represent the most widely used class of enzymes in biotechnological
38 applications and organic chemistry. Phylogenetic analysis and comparison of residues around
39 GxSxG motif provided an insight to the diversity among bacterial lipases. A variety of para-
40 Nitrophenyl (p-NP) esters having C_2 to C_{16} (p-NP acetate to p-NP palmitate) in their fatty acid
41 side chain can be hydrolyzed by bacterial lipases. Large heterogeneity has been observed in
42 molecular and catalytic characteristics of lipases including molecular mass; 19–96 kDa, K_m;
43 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
44 conditions of their working temperature and pH have been stated 15–70 °C and 5.0–10.8,
45 respectively and are strongly associated with the type and growth conditions of bacteria. Surface
46 hydrophobicity, enzyme activity, stability in organic solvents and at high temperature,
47 proteolytic resistance and substrate tolerance are the properties of bacterial lipases that have been
48 improved by engineering. Bacterial lipases have been extensively studied during last decade.
49 However, their wider applications demand a detailed review on purification, catalytic
50 characterization and applications of lipases.

51 Keywords: Glycerol ester hydrolase, Triacylglycerol acylhydrolase, Phylogenetic, Human health,
52 Physiochemical, Kinetics.

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71 **1. Introduction**

72 Significant rising concern in the field of enzymology on account of wider applications of
73 enzymes in various chemical processes has been increased since a few decades (Pliego et al.,
74 2015). Due to versatile applications, lipases are the third most abundantly used enzymes after
75 proteases and amylases (carbohydrases) (Ülker et al., 2011). Lipase (E.C.3.1.1.3) is also known
76 as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase and belongs to the class

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77 of enzymes that catalyze the hydrolysis reactions (hydrolases). Lipase catalyzes the hydrolysis of
78 triglycerides converting them to glycerol and fatty acids in an oil-water interface. Lipases also
79 have a property to reverse this reaction in an aqueous and non-aqueous media (Faouzi et al.,
80 2015b; Lee et al., 2015; Priji et al., 2015; Ramos-Sanchez et al., 2015; Ullah et al., 2015). Some
81 lipases show enantioselective properties and used to catalyze the processes of esterification,
82 interesterification, transesterification, acidolysis and aminolysis (Hasan et al., 2009). The
83 substrates of lipases (long chain triacylglycerols) are insoluble in water, hence, these are first
84 dissolved in organic solvents followed by mixing with buffer (two-phase system). However,
85 lipases are soluble in water and can catalyze their reactions in two types of systems including
86 aqueous and organic medium. Organic solvents may denature and cause conformational changes
87 in the lipase structure and hence influence their functional and catalytic activities (Guo et al.,
88 2015).

89 Lipases are ubiquitous enzymes (Priji et al., 2015), belong to α/β hydrolase fold super-
90 family (Kapoor and Gupta, 2012) and have a network of hydrogen bonds at their active site
91 containing triad of Ser, Asp (Glu) and His (Faouzi et al., 2015b; Farrokh et al., 2014; Thakur et
92 al., 2014). Lipase catalyzed transesterification for the production of biodiesel is an efficient,
93 energy-saving, and environment friendly process and is a promising alternative to the
94 conventional chemical catalysis (Fjerbaek et al., 2009). Lipases are substrate specific enzymes
95 and have properties like chemo-, region-, stereo-specificity and ability to catalyze heterogeneous
96 reactions both in water soluble and water insoluble systems. On account of their wider catalytic
97 properties, lipases are extensively used as biocatalysts in different industries like agrochemical,
98 pharmaceutical, detergent, tanning, food and surfactant producing industries (Ananthi et al.,
99 2014; Iftikhar et al., 2012; Kumar et al., 2012; Thakur et al., 2014).

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

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

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

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

113 Lipases need no co-factor for their activity and remain active in organic solvents (Lee et
114 al., 2015; Ullah et al., 2015). Consumption of all monoglycerides, diglycerides, triglycerides and
115 free fatty acids in the process of transesterification, high production in non-aqueous media, low
116 reaction time and resistance to low pH are some of the properties which make lipases more
117 desirable biocatalysts (Ashfaq, 2015). Lipases are of plant, animal, and microbial origin, but
118 microbial lipases are produced at industrial level and represent the most widely used class of
119 enzymes in biotechnological applications and organic chemistry due to higher catalytic activity,
120 seasonal changes independent production, ease in genetic manipulation for desired
121 characteristics, production in bulk quantity and use of cheaper growth culture media (Dey et al.,
122 2014; Lee et al., 2015; Priji et al., 2015; Ullah et al., 2015).

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

132 **2. Lipase producer bacterial strains**

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

137 **Table 1**
138 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 Dhundale, 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., 2012a)
Bacillus sphaericus	(Joseph and Ramteke, 2013)	*Pseudomonas fluorescence*	(Kumar et al., 2012)
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., 2016)
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)

139 **3. Phylogeny and genetic basis of lipase producing bacteria**

140 Comparison among the sequences of different bacterial lipases highlighted the fact that
141 they appear to be quite variable. Lipases carry a wide diversity of properties both on biochemical
142 or molecular level. The only known conserved sequence common among all lipases is Gly-X-
143 Ser-X-Gly pentapeptide, which encloses the active site serine residue. For molecular
144 phylogenetic analysis of different lipase producing bacteria (Table-1), sequences of 42 proteins
145 representing 7 diverse phylogenetic clusters are used here (Fig.1). Further sub-clusters were

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146 observed in each group on the basis of closeness in the sequences of lipase proteins. The sub-
147 clusters showed further branching based on the closeness of lipase protein sequences and
148 revealed significant diversity among lipase producing bacteria. Analysis of residues around
149 GxSxG motif also demonstrated existence of diversity among different lipase producing bacteria
150 and provided an insight to the closeness of lipases of different bacteria based on amino acid
151 sequences. Such information could be utilized for the designing of probe or primers for studying
152 lipase genes of diverse groups and classification of newly identified lipases (da Silva et al., 2013;
153 Leathers et al., 2013; Masomian et al., 2016).

154 These lipases are classified on the basis of their biochemical properties. However, in
155 current era of genomics and bioinformatics, significant number of new lipases have been
156 discovered that deviate from existing criteria of classification (Arpigny and Jaeger, 1999; Eggert
157 et al., 2001; Jaeger et al., 1994). Moreover, the ever increasing information about protein
158 structure by using techniques like X-ray crystallography or NMR spectroscopy, has added
159 considerable uncertainty about the usefulness of current classification system (Castilla et al.,
160 2017; Masomian et al., 2016). Therefore, a comprehensive attempt is required in this regard to
161 provide a more representative criteria for the classification of lipases.

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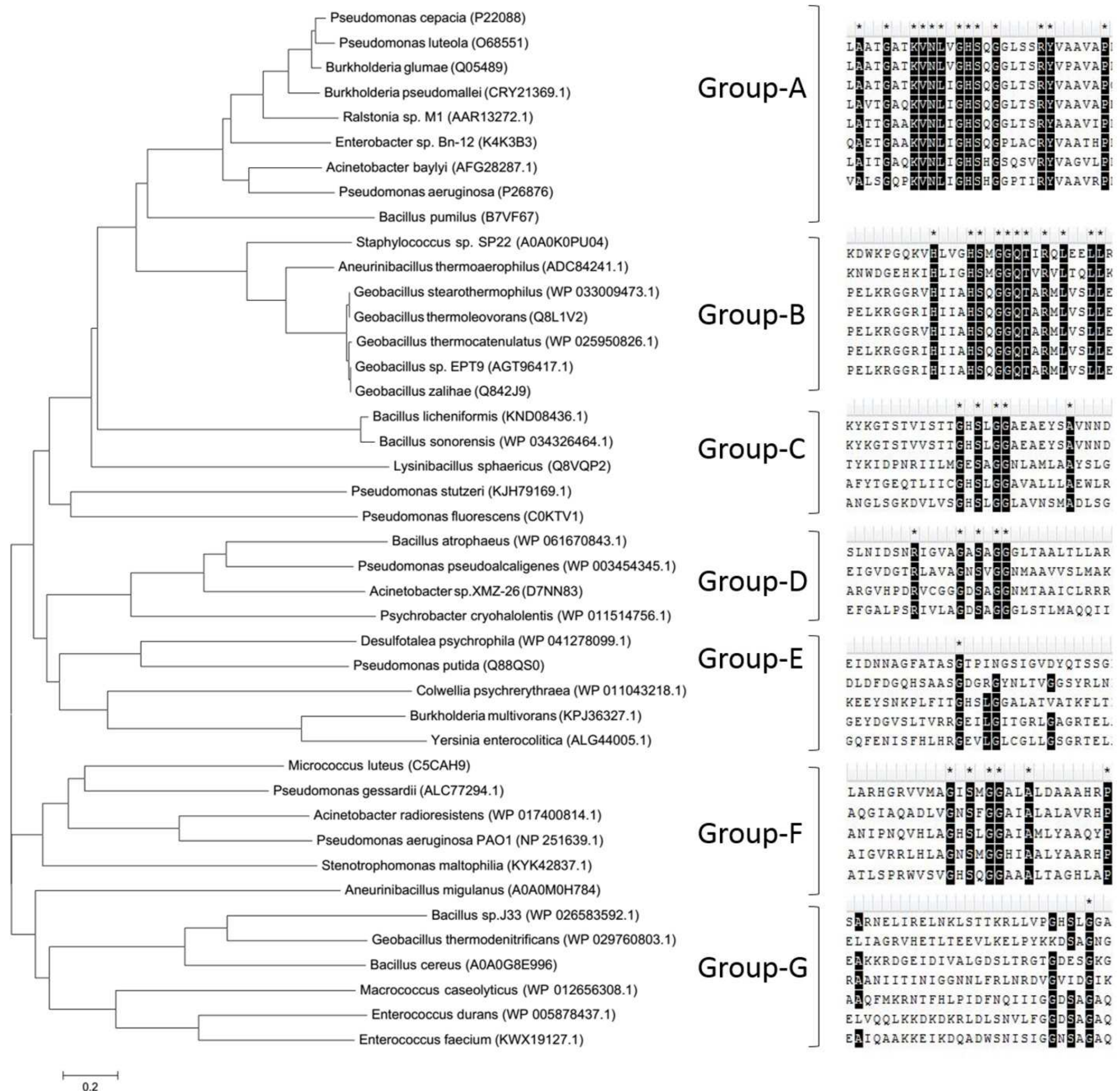

Figure 1. Phylogenetic analysis and multiple sequence alignment showing the relationship among different lipase producing bacteria. Rooted phylogenetic tree was built by using the Neighbor-Joining method [75]. The bootstrap consensus tree was inferred from 1000 replicates. The optimal tree with the sum 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.

175 **4. Purification of bacterial lipases**

176 The purpose of purification is not only to isolate the enzymes from contaminants but also
177 to improve their activity, stability and shelf life. Structural and conformational studies can be
178 conducted after the proteins are purified up to homogeneity level (Nadeem et al., 2009, 2015a).
179 Kinetic and thermodynamic mechanism of lipases for substrate hydrolysis, transesterification
180 reaction and structure-function relationship can only be established for purified lipases.
181 Moreover, purified lipases are needed to make important formulations for industrial and
182 medicinal uses. Purification is a key step that is performed to interpret the actual function of a
183 specific enzyme. Bacterial cells are removed from culture broth after the fermentation process to
184 get extracellular lipases. The cell free extract is then concentrated by extraction with organic
185 solvents, ultrafiltration or by ammonium sulphate precipitation. Ammonium sulphate
186 precipitation is mostly used during early stages of purification, considered as a crude separation
187 step and is followed by a combination of chromatographic steps (Saxena et al., 2003). Broth or
188 cell culture is passed through various stages starting from salt precipitation to column
189 chromatography depending upon the nature of the proteins and desired level of purification.
190 Various chromatographic techniques including anion exchange, cation exchange and size
191 exclusion chromatography are used for the purification of lipases. Data from various studies
192 suggested that lipases had been purified from 2.4 to 500 fold purification with 10.3 to 36%
193 overall yield (Table-2).

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203 **Table-2**
204 Purification strategies recently used for various studies of bacterial lipases.
205

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 and ion-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)
Geobacillus stearothermophilus 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^{TM} 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., 2012)
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*	Immobilized metal affinity chromatography (IMAC)	14.6 /63.2	(Ebrahimpour et al., 2011)

TOP10)			
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)

206 NR= Not Reported

207 ## 5. Characterization of bacterial lipases

208 ### *5.1 Physiochemical properties of bacterial lipases*

209 Physiochemical properties of twenty seven recently studied (2010 to 2017) bacterial
210 lipases are summarized in table-3. It can be concluded from these studies that all bacterial lipases
211 except that of *Halobacillus* sp. strain LY5 (Xin et al., 2012) and *Pseudomonas gessardii*
212 (Ramani et al., 2010) have molar masses less than 70 kDa. A minimum molar mass of 19 and
213 19.2 kDa was observed for lipases from *Bacillus stratosphericus* and *Enterococcus faecium* by
214 Gricajeva *et al*. (2016) and Ramakrishnan *et al*. (2016), respectively. Lipases obtained from
215 *Pseudomonas gessardii*, *Spirulina platensis* and *Bacillus pumilus* RK31worked optimally at
216 acidic pH, while all other bacterial lipases worked at alkaline pH. *Cohnella* sp. A01 lipase
217 studied by Golaki *et al.* (2015) showed maximum activity at 70 °C, while all other bacterial
218 lipases showed optimal activity below 70 °C. Zheng *et al.* (2011) reported a minimum (15 °C)
219 optimum temperature for cold active *Acinetobacter* sp. XMZ-26 lipase.

220

221
222 **Table 3**
223 Physiochemical properties of recently studied bacterial lipases.
224

Strain	Mass. (kDa)	pH Opt.	Temp. Opt. (ºC)	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., 2012)
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)

225 *5.2 Kinetic properties of bacterial lipases for substrate hydrolysis*

226 A variety of para-Nitrophenyl (p-NP) esters having C_2 to C_{16} (p-NP acetate to p-NP
227 palmitate) in their fatty acid side chain can be hydrolyzed by bacterial lipases. Kinetics of
228 substrate hydrolysis for different esters are summarized in table-4. The affinity of a lipase for
229 substrate hydrolysis is determined by Michaelis constant (K_m) that is the substrate concentration
230 at which the rate of reaction is half of the maximum rate (V_{max}). V_{max} is the maximum rate when
231 an enzyme is fully saturated with substrate concentration (Nadeem et al., 2015b). *Streptomyces*

232 *lividans* lipase reported by Wang *et al*. (2016) have high affinity (K_m = 0.0064 mM) for the
233 substrate (p-NP caproate) but have the lowest value of catalytic constant (K_{cat} = 0.1665 s^{-1}) and
234 specificity constant (K_{cat}/K_m = 26.02 s^{-1}/mM). However, *Acinetobacter* sp. XMZ-26 lipase
235 studied by Zheng *et al*. (2011) have high affinity (K_m = 0.075 mM) for the substrate (p-NP
236 octanoate) as well as high catalytic constant (K_{cat} = 560.52 s^{-1}) and highest specificity constant
237 (K_{cat}/K_m = 7377 s^{-1}/mM). Catalytic properties of the enzymes are dependent on various factors
238 including type and concentration of the substrate, concentration of enzyme, pH and temperature.

239 **Table 4**
240 Kinetic properties of bacterial lipases for substrate hydrolysis.
241

Strain	Substrate	V_{max} (µM 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	5.24×10^5	16.58	NR	10^4	621	(Ji et al., 2015)
Ralstonia sp.	p-NP palmitate	1.01×10^5	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)

242 p-NP = para-Nitrophenyl, NR = Not Reported

243 **6. Engineering/Modification of bacterial lipases**

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

251 Protein engineering involves two major strategies: rational design and directed evolution
252 (Bornscheuer, 2013, 2008). Rational design requires two types of information about proteins
253 which include data of three-dimensional structure and relation between protein structure and
254 function (Lan et al., 2015). Contrary to rational design, such information is not required for
255 directed evolution studies (Porter et al., 2016), which involves random mutagenesis of the target
256 gene, generation of mutant library and screening to recognize a variant having desired
257 characteristics (Bornscheuer, 2008). The generation of mutant library involves two approaches: a
258 non-recombining and a recombining evolution. In the first approach, point mutations are
259 introduced in a target gene. For this purpose different techniques including UV irradiation,
260 chemical mutagenesis or error-prone polymerase chain reaction (epPCR) are used. The
261 recombining evolution is based on recombination principle. As a first step, a pool of recombinant
262 chimera is generated by shuffling and reconstruction of several parental genes. The sources of
263 parental genes may comprise either mutants of a single gene (generated by other mutagenesis

264 methods) or naturally occurring homologs of a gene family (Bornscheuer, 2008; Lülsdorf et al.,
265 2015; Porter et al., 2016). The recombinant variants of gene are cloned followed by
266 transformation and expression in a host system. At the end, a high-throughput screening is
267 executed to isolate a variant with the best desired characteristics. Both the recombining and non-
268 recombining evolution methods are performed *in vivo* (directly on colonies growing on agar
269 plates) or *in vitro* (on cultures of individual clones) (Hasan et al., 2009).

270 Molecular engineering technologies have been applied to better understand the catalytic
271 mechanism or have been used to improve the specific properties of microbial lipases (Table-5).
272 Recently, protein engineering for thermo-stable (Ahmad and Rao, 2009; Gumulya and Reetz,
273 2011; Khurana et al., 2011; Shih and Pan, 2011; Wu et al., 2009) and organic solvent-tolerant
274 lipases (Dror et al., 2014; Kawata and Ogino, 2010; Monsef-Shokri et al., 2014; Reetz et al.,
275 2010; Yedavalli and Rao, 2013) has been the focus of research. Improvement of properties like
276 inherent stability against high temperatures and capacity to tolerate harsh organic solvents have
277 substantial future in an array of synthetic reactions in industry (Masomian et al., 2010).
278 Similarly, the bio-catalytic properties of lipases serve as an important environment friendly
279 substitute to the conventional chemical approaches. Though, lipase-catalyzed processes hold
280 eminent commercial worth, yet the use of lipases is restricted owing to low yields, stumpy
281 reproducibility, and inconsistent optimal performance in native form. Protein engineering by
282 recombinant DNA technology is providing appropriate means to overcome these shortcomings
283 and to produce robust enzyme catalysts at high yields (Ema et al., 2012; Knapp et al., 2016;
284 Mohammadi et al., 2016; Wi et al., 2014). With the increasing understanding of the mechanisms
285 for the regulation of gene expression, different expression systems have currently been optimized
286 for improved functional characteristics and new hyper-producing strains (Knapp et al., 2016). An

287 increased yield of recombinant lipases is important on industrial scale because it is required for
288 increased volumetric productivity, reduced downstream purification costs, and much purer
289 resulting crude enzyme.

290 **Table 5**
291 Improved properties of bacterial lipases through engineering.
292

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-guided consensus	(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)

293 **7. Applications of bacterial lipases**

294 Ease of availability (due to production at industrial level), ability to work in
295 heterogeneous media, interfacial activation phenomenon and large scale substrate specificity are
296 the factors due to which lipases are largely used in various industrial applications (Hasan et al.,

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297 2006; Kapoor and Gupta, 2012). Moreover, being non-toxic and eco-friendly, lipase are
298 considered more suitable as compared to other chemical or synthetic catalysts. Therefore, widely
299 used in food, dairy, flavor, detergent, pharmaceuticals, biofuels and cosmetics industries. Esters
300 having natural flavor are formed by esterification and trans-esterification using lipases
301 (Rajendran et al., 2009). Detergent industries consume almost 1000 tons of lipases every year in
302 hydrolysis reaction to remove oil stains (Hasan et al., 2006; Parra et al., 2015).

303 *7.1 Food industry*

304 Lipases are used for the hydrolysis of milk fat, cheese ripening, flavor enrichment and
305 lipolysis of butter fat in dairy industry (Adrio and Demain, 2014; Boonmahome and
306 Mongkolthanaruk, 2013; Farrokh et al., 2014; Ferreira-Dias et al., 2013; Konkit and Kim, 2016;
307 Rigo et al., 2010; Sirisha et al., 2010; Ullah et al., 2015). Halo-tolerant lipase obtained from
308 *Lactobacillus plantarum* is used in the synthesis of different fermented food products such as
309 sour dough, olives, vegetable sausages and cheese (Esteban-Torres et al., 2015). *Pseudomonas*
310 lipase was considered to be useful in food processing and oil manufacturing. Castor oil is a
311 unique vegetable oil that contains high amounts (90%) of a hydroxy monounsaturated fatty acid
312 named ricinoleic acid. This industrially important acid can be obtained by hydrolysis of castor oil
313 using more efficient lipase-catalyzed process (Goswami et al., 2012). Cocoa butter equivalents
314 are produced by lipase catalyzed inter-esterification of natural triglycerides, such as middle
315 fraction of palm oil or sunflower. Moreover, lipase can be used to enhance the shelf life, flavors
316 and rheological properties of fruit juices, soups, sauces, cheese and baked foods. The shelf life of
317 different bakery products and their softness can be improved with lipases. Softness of noodles
318 can also be enhanced with the help of lipases. Phospholipases also found to be used in the
319 treatment of egg yolk for the manufacturing of mayonnaise and various emulsifiers (Ray, 2012).

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320 *Acinetobacter* sp. EH28 lipase is used to make flavor esters like ethyl butyrate, ethyl valerate and
321 ethyl caprylate in organic solvents. Ethyl caprylate, which has a fruity-flowery fragrance, is
322 applied to give different fruity flavors like peach, apple, banana and pineapple (Ahmed et al.,
323 2010). Lipases are also applied for flavor modification and gelling in fish flesh for protein
324 polymerization (Joseph et al., 2007). One important application of lipase is in hydrolysis of
325 vegetable oil for the generation of free acids which are further used in food, soap and biomedical
326 industries.

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

339 *7.2 Pharmaceuticals*

340 A thermo-stable lipase obtained from *Acinetobacter baylyi* also resistant to organic
341 solvents works as an efficient catalyst in bioenergy, pharmaceutical industries and for trans-
342 esterification of palm oil to FAMEs (Uttatree et al., 2010). Moreover, lipases also have medicinal

343 uses such as skin scalp disease and hair loss can be treated by using this enzyme (Sangeetha,
344 2011). Cold active lipases are used to make different useful chemical compounds such as aryl
345 aliphatic glycolipids, citronellol laurate from citronellol and lauric acid, and ethyl esterification
346 of docosahexaenoic acid to ethyl docosahexaenoate. *Bacillus* lipases showed selectivity to the
347 fatty acid chain length of an ester, and few enzymes display positional specificity. Due to these
348 properties, *Bacillus* lipases can be used in pharmaceutical industries for the synthesis of
349 enantiopure compounds (Guncheva and Zhiryakova, 2011). Antioxidants such as tyrosol acetate,
350 propyl gallate and eugenol benzoate are formed by using *Staphylococcus* lipase (Horchani et al.,
351 2012).

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

360 *7.3 Biofuels*

361 Trans-esterification process catalyzed by lipase is performed in the presence of fatty acids
362 and short chain alcohol usually methanol (Lotti et al., 2015). Methanol and waste cooking oil are
363 converted into biodiesel and glycerol in the presence of lipases (Karmee et al., 2015).
364 *Acinetobacter venetianus* RAG-1 lipase is used for the production of biodiesel by trans-
365 esterification (Boonmahome and Mongkolthanaruk, 2013). Micro crystals of K_2SO_4 coated with

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366 lipase are used as an alternative to chemical catalysts for the production of biodiesel (Sirisha et
367 al., 2010; Zheng et al., 2012b). Alkali stable lipases are more useful in the production of
368 biodiesel because alkaline pH is useful to improve oil solubility and homogeneity of the reaction
369 mixture giving high trans-esterification rates and biodiesel production (Christopher et al., 2015;
370 Li et al., 2016b). Lipase trans-esterification reduced the downstream processing cost in biodiesel
371 production (Hegde et al., 2015). *Burkholderia cepacia* lipase immobilized on NKA resin is used
372 to make a high value biocatalyst and for the production of biodiesel (Liu et al., 2011). In fact,
373 cross linking of *Burkholderia cepacia* lipase with glutaraldehyde and then entrapment in a hybrid
374 matrix of equal proportions of alginate and k-carrageenan used for biodiesel production. This
375 immobilized lipase remained active after six cycles of reuse. So this is a good source for
376 biodiesel production industry (Abdulla and Ravindra, 2013). Lipase from *Ralstonia* sp. catalyzed
377 the production of biodiesel in the presence of soya bean and palm oil but more efficiently in the
378 latter case at pH 8 with 5% methanol and 20% water content (Yoo et al., 2011). Moreover,
379 lipases are used as biocatalysts to make biodegradable polymers such as 1-butyl oleate, which is
380 used in biodiesel to reduce the viscosity in winter season. Tri-methylolpropane esters originated
381 from lipases are used as lubricants. Lipase isolated from *Bacillus* sp. showed high activity
382 towards oleic rich oils. Lipase immobilized on celite could retain 90% lipase activity after eight
383 cycles. Trans-esterification of oil using the immobilized lipase obtained from *Botryococcus* sp.
384 resulted in 80% yield of fatty acid methyl esters which had good properties for use as biodiesel
385 (Sivaramakrishnan and Incharoensakdi, 2016).

386 A new lipase SL-4 from *Burkholderia ubonensis* was employed to catalyze soybean oil
387 for biodiesel production, the liquid lipase SL-4 could secure a conversion ratio of 92.24% in a
388 solvent-free system. That resulted in a new thermo-solvent-stable lipase possessing an attractive

389 potential for biotechnological applications as biocatalyst, especially for biodiesel production
390 (Yang et al., 2016).

391 *7.4 Detergents*

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

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

407 *7.5 Other applications of lipases*

408 Bacterial lipases are also applied in the formation of biopolymers. *B. multivorans* V2 was
409 observed to produce solvent-tolerant lipase used for the synthesis of ethyl butyrate ester in non-
410 aqueous environment (Sarethy et al., 2011) . Omega- 3 poly-unsaturated fatty acids are prepared
411 by alcoholysis of cod liver oil in the presence of *Pseudomonas* lipase (Pallavi et al., 2014).

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412 Lipase obtained from *Pseudomonas* ADT3 was found useful in leather and chemical industries
413 (Dey et al., 2014). Lipases are also used in the bioremediation of different industrial and
414 municipal wastes (Shafqat et al., 2015).

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

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

432 **8. Conclusion**

433 Bacterial lipases are an important group of enzymes that offer enormous potential for
434 various applications, and there is considerable interest in identifying and developing novel

435 bacterial lipases. Detailed study of the latest literature indicates that lipases are one of the most
436 produced bacterial enzymes. Many researchers worldwide are conducting their studies on
437 isolation, screening and optimizing the conditions for bacterial strains to gain maximum
438 production of lipases. Versatile application of lipases is the justification of all these efforts.
439 Although the cold active and thermo tolerant bacterial strains and their products (lipases) have
440 been identified but there is a large vacuum for engineered lipases with significant characteristics
441 for specific applications. The properties of lipases that need to be improved are stability and
442 turnover under application conditions. They need to be robust and versatile with respect to the
443 range of substrates they can act on, but at the same time they should have a high specificity for
444 the reactions they catalyze.

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