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BIOCATALYSIS

Application of Bacterial Thermostable Lipolytic Enzymes in the Modern Biotechnological Processes: A Review

Yu. V. Samoylova[a], *, K. N. Sorokina[a], **, A. V. Piligaev[a], *, and V. N. Parmon[a], ******

[a] *Boreskov Institute of Catalysis, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*

**e-mail: samoylova.jv@catalysis.ru*

***e-mail: sorokina@catalysis.ru*

****e-mail: piligaev@catalysis.ru*

*****e-mail: parmon@catalysis.ru*

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Abstract—Review focuses on the modern applications of bacterial lipolytic enzymes in biotechnology and covers the scope of their properties including their activity and functional stability at different temperatures, pH, substrate specificity, and activity in the presence of different chemicals. The recent data on the production of genetically engineered strains producing the bacterial lipolytic enzymes and approaches to improving their productivity are presented. The applications of bacterial lipases in biotechnological processes used in the production of biofuel, chemicals and detergents, in the food industry, and in wastewater treatment are considered.

Keywords: lipase, esterase, bacterial enzymes, immobilization, biocatalysis

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INTRODUCTION

The 21st century has been marked by the rapid development of biotechnology. Enzymatic catalysis has a special place in this field, due to the diversity and specific properties of enzymes [1–3]. The most important enzymes for chemical, food, pharmaceutical and fuel industries include hydrolases (lipases and esterases) that are used in hydrolysis and esterification reactions, especially in the immobilized form. The significant progress has been achieved in application of lipases and esterases as the part of biocatalysts for the synthesis of optically pure compounds, in biofuel production, and as the detergent additives [7, 8].

Lipases belong to the superfamily of α/β hydrolases with the active center formed by Ser-His-Asp/Glu amino acid residues and covered with the *lid*-domain. It has been shown that thermostable lipases have the large *lid*-domain with two or more α-helices, while the mesophilic lipases are characterized by smaller structures consisting of a loop or an α-helix [9]. This domain allows lipases to exist in two conformations: open and closed. At an oil–water interface, the enzyme is activated through the conformational changes accompanied by a shift of the *lid*-domain, resulting in opening of the substrate-binding pocket [10]. During the substrate hydrolysis, serine residue acts as a nucleophilic donor of hydroxyl groups and chemical compounds act as the donors of the acyl groups. This explains the broad substrate specificity of

these enzymes, including specificity to the hydrolysis of ester bonds in the certain positions of triacylglycerides [11].

In contrast to lipases, esterases have higher specificity to the residues with the length of less than C10 [12] and do not require interface activation. Most of the known esterases are carboxylesterases with α/β-structure. The enzymes consist of six α-helices surrounded by loops and eight β-folds. The active center of the enzymes is formed by the Ser, Asp and His residues; in addition, there is a specific structure referred to as a "nucleophilic elbow," which contains a nucleophilic group. Meanwhile, the neighboring amino acid residues participate in the formation of other structures: oxyanion holes, whose main function is to stabilize the negative charge of the substrate during the nucleophilic attack [13].

The diverse properties of lipolytic enzymes generally allow classifying them according to their origin, structure, and the catalyzed reactions. They are currently classified as: carboxylesterases (EC 3.1.1.1), galactolipases (EC 3.1.1.26), triacylglycerol lipases (EC 3.1.1.3), acylglycerol lipases (EC 3.1.1.23), sterol esterases (EC 3.1.1.13), arylesterases (EC 3.1.1.2), and a number of other enzymes [12].

NATIVE PRODUCERS OF LIPOLYTIC ENZYMES

Lipolytic enzymes are widespread in nature; they are found in bacteria, archaea, plants, and fungi [12]. Extracellular lipases are used most extensively, since they can be easily isolated from the culture medium. Although lipolytic enzymes are produced by many microorganisms, the recombinant strains are predominantly used for their production as they satisfy the requirements of the manufacturing processes. Improved native producers are used at a low expression of lipolytic enzymes of one organism in the cells of another organism (heterologous expression) [14, 15].

Industrial lipolytic enzymes are now derived mainly from the filamentous fungi, due to the sufficient levels of expression attained for some strains. For example, *Aspergillus oryzae* has been modifyed to produce the *sn*-1,3-specific thermostable lipase from *Thermomyces lanuginosus*. If the producer strain is a native bacterial strain, its properties (including recombinant enzyme production) are first improved through selection. Under both laboratory and industrial conditions, lipolytic bacterial enzymes are now derived from native producers belonging to the genera *Bacillus*, *Geobacillus*, *Achromobacter*, *Burkholderia*, *Pseudomonas*, *Chromobacterium*, *Streptomyces*, and *Arthobacter* [4, 16, 17]. The most commonly used bacteria belong to the genus *Bacillus*, which is widespread in nature and produce lipases and esterases used for the production of biodiesel fuel [18] detergents [19], chemical synthesis [20] and water treatment for of insecticides degradation [21]. These enzymes have high functional stability at high temperatures in a wide range of pH, and in the presence of various chemicals. The properties and applications of some lipolytic enzymes from bacteria of the genus *Bacillus* are listed in Table 1.

The enzymes of g. *Bacillus* have high functional stability and temperature optimum at 55–70°C, allowing to use them in different processes. For example, the *n*-hexane and *tret*-butanol tolerant lipase from the *Bacillus* sp. with an optimum temperature of 55°C has been used for the production of biodiesel from lipids of the microalga *Oedogonium* sp. with a 76% yield of fatty acid methyl esters (FAMEs) after 40 h [26]. The high functional stability and stereospecificity of bacterial lipases toward chemicals makes them suitable for application in chemical synthesis. The intensive studies on the properties of bacterial enzymes in reactions of stereospecific synthesis has been on the hydrolysis of (*R*,*S*)-2-(3-benzoylphenyl)propionic and (*R*,*S*)-2-(4-isobutylphenyl)propionic acid ethyl esters. Lipolytic enzymes of the bacteria *A. flavithermus* and *B. gelatini* KACC 12197 (Table 1) provide highly efficient chiral resolution of the products of hydrolysis of ketoprofen and ibuprofen. It should also be noted that only one bacterial lipolytic enzyme (recombinant carboxylesterase D1CarE5 from *A. tengchongensis*) has been successfully used for the biodegradation of insecticides, e.g., malathion [27].

PROPERTIES OF BACTERIAL LIPOLYTIC ENZYMES

Activity and Stability of Bacterial Lipases and Esterases at Different Temperatures

High temperatures are often used in industrial processes, since they increase the rate of reaction and improve product yield by shifting the balance in endothermic reactions. The high temperature also reduces the contamination of culture medium with the mesophilic bacteria (the associated microflora) [30]. High-temperature reactions require use of enzymes with high activity and functional thermostability. The currently produced commercial thermostable lipolytic enzymes of filamentous fungi *T. lanuginosus, Rhizomucor miehei* and *Pseudozyma antarctica* (*Candida antarctica*) have optimum activity at 37–50°C. On the contrary there are the thermostable bacterial lipases of *Burkholderia cepacia* and *Pseudomonas fluorescens* with higher temperature optimum (above 50°C) [12]. Thermostable lipolytic bacterial enzymes from other microorganisms are used less frequently, though they might have much higher functional thermostability. Bacteria isolated from extreme environment (including hot springs with temperatures of 50–100°C) produce enzymes under laboratory conditions that are active at temperatures of up to 110°C [31]. Among the thermostable lipases functionally stable at 50–75°C the enzymes of the thermophilic bacteria of the genera

Table 1. Properties of lipolytic enzymes produced by bacteria of the genus *Bacillus*

Producer	Enzyme properties	Application	Reference
Bacillus licheniformis B42 (lipase)	pH 9–9.5; 50–60°C	Production of detergents	[22]
Anoxybacillus flavithermus (recombinant lipase/esterase Est/Lip)	25 kDa; pH 6.5–8; 60–65°C. At pH 8.0 and 60 °C, $t_{1/2} \approx 5$ h. Enzyme is active in the presence of DMSO, acetonitrile, and methanol. Substrate specificity: *p*NPC4. Enantioselective to *S*-enantiomers of (*R*,*S*)-1-phenylethyal acetate and (*R*,*S*)1-(benzofuran-3-yl)-3-ethyoxy-3-oxopropyl decanoate	Chemical synthesis	[23]
Bacillus gelatini KACC 12197 (recombinant lipase)	42 kDa; pH 10; 65°C. Retains more than 90% of its initial activity at 65°C, pH 10, for 180 min. Substrate specificity: *p*NPC2, *p*NPC4. Enantioselectivity towards (*S*)-ketoprofen ethyl ester	Chemical synthesis	[24]
Bacillus sp. (recombinant lipase L2)	43.2 kDa; pH 9; 70°C. At pH 9.0 and 60°C, $t_{1/2} \approx 2$ h	Detergent production, food industry	[25]
Bacillus sp. (lipase)	45 kDa; pH 7; 55°C. Active in the presence of *n*-hexane and *tret*-butanol	Biodiesel fuel production	[26]
Alicyclobacillus tengchongensis (recombinant carboxylesterase D1CarE5)	58 kDa; pH 7; 60°C	Biodegradation of insecticides (malathion)	[27]
Bacillus thuringiensis	No data	Biodegradation of insecticides (malathion)	[28]
Bacillus sp.S23 (lipase)	45 kDa	Biodiesel fuel production	[29]

Bacillus and *Geobacillus* are extensively studied [32]. The recently isolated bacteria belonging to *Geobacillus kaue* and *Geobacillus kaustophilus* have an optimum temperature of 80°C and are stable at 90°C for 30 min [33]. Thermostable esterases LipA and LipB of the thermophilic bacterium *Thermosyntropha lipolytica* have maximum activity at 96°C, which is currently the highest for the lipolytic enzymes [34]. Lipase of the bacterium *T. lipolytica* also has a high functional thermostability. In [35], it was shown that the enzyme retained 100% of its initial activity for 180 min at 70°C; 53.5% and 36% lipase activity was maintained for 120 min at 80°C and for 60 min at 90°C, respectively. Computer analysis of the structure of thermostable lipases from bacteria of the genus *Geobacillus* revealed the presence of coordinated amino acid substitutions in a group of lipases with high functional thermostability (with a half-inactivation time of more than 1000 min): V198A, Q203E, V204I, Q217E and V294I, P306A, T307A, D312S, R313H, E316G, V324I, S334N, A343T. Stabilization of the *lid*-domain in the 198A-217E region is observed in lipases with the maximum functional thermostability [36].

The optimum activity of bacterial lipases predominately lies in the range of 30–60°C [37]. It has been shown that the thermostability of bacterial lipolytic enzymes increases after addition of various chemicals. For example, the activity of lipases of some bacteria of the genus *Bacillus* in the presence of ethylene glycol, sorbitol, or glycerol is retained at 70°C after 150 min of incubation [38].

Activity and Stability of Bacterial Lipases and Esterases at Different pH

Enzymes with neutral or alkaline optimum are commonly used for the production of biodiesel fuel and modified fats. It has been shown that the bacterial lipases have maximum activity in neutral [26] or alkaline [39] media (pH 6.0–11.0). For example, alkaline lipases isolated from *B. licheniformis* [40], *Bacillus stratosphericus* L1 [39] and *Bacillus* sp. L2 [25] have optimum pH in the range of 9.0 to 11.0. Lipases of the bacteria *Bacillus stearothermophilus* SB-1, *Bacillus atrophaeus* SB-2 and *B. licheniformis* SB-3 are active at pH 3.0–12.0 [41]. Lipases with the optimum activity in acidic media are less frequent. For example, the optimum pH in the range of 3.5–4.8 is typical for lipases of *Pseudomonas gessardii* [42] and *P. fluorescens* SIK W1 [43]. The diversity of lipolytic enzymes promotes their application in the various reactions requiring different pH.

Stability of Bacterial Lipases and Esterases in Organic Solvents

Functional stability is an important property of enzymes in organic solvents, since the presence of organic solvents in a reaction mixture can shift the balance of reaction toward the formation of products like fatty acid esters during oil methanolysis. Addition of organic solvents increases the solubility of substrates (e.g., plant oils), reduces formation of by-products, and prevents microbial contamination of the product [44].

A number of studies have shown that with the few exceptions bacterial lipases are highly stable in aqueous media containing organic solvents (e.g., methanol, ethanol, isopropanol, and *n*-hexane). For example, addition of methanol at the concentration of 30% (v/v) increases the activity of the lipase of the bacterium *Bacillus thermocatenulatus* by 18% [45], while addition of *n*-hexane (up to 60% (v/v) decreases the activity of the lipase of *Bacillus* sp. [46]. It was shown that the activity of lipase of strain *Bacillus sphaericus* 205y in the presence of *n*-hexane and *p*–xylene increases by 3.5 and 2.9 times, respectively, as compared to the enzyme activity without solvents [47]. The activity of the lipase TSLip1 of the bacterium *T. lipolytica* decreases in the presence of organic solvents: the enzyme retains more than 80% of activity in the presence of 10% (v/v) methanol, but its activity is almost completely inhibited in the presence of 30% (v/v) methanol or 20% (v/v) ethanol [48].

Effect of Metal Ions on the Properties of Bacterial Lipases and Esterases

It was shown that divalent cations (e.g., Ca^{2+}) can increase the activity of lipases and esterases, due to the formation of calcium salts of long-chain fatty acids [49]. Ca^{2+} ions increase the activity of lipases of *Geobacillus thermodenitrificans* IBRL-nra [50], *Pseudomonas aeruginosa* [51], and *T. lipolytica* [34]. On the other hand, it was shown that calcium ions can reduce lipase activity, as was observed for the lipase of *P. aeruginosa* 10145 [52]. In addition to Ca^{2+} ions, lipase activity is reduced by Co^{2+}, Hg^{2+}, and Sn^{2+} [52]; and to a lesser extent by Mg^{2+} and Zn^{2+} [53]. Fe^{3+} ions have the opposite effect: they increase the lipase activity of *A. calcoacelicus* LP009 by 20% [54].

Substrate Specificity of Bacterial Lipases and Esterases

Substrate specificity is one of the most important properties of enzymes. According to this, bacterial lipases are classified as nonspecific, regiospecific, and specific to fatty acid with different lengths of residues (short-chain C_2–C_7, middle-chain C_8–C_{12}, long-chain $>C_{12}$). Nonspecific lipolytic enzymes, including those produced by *Chromobacteriuin viscusum, Staphylococcus aureus, Staphylococcus hyicus* [55], and *Corynebacterium acnes* [56], hydrolyze triacylglycerides, to mono- and diglycerides, fatty acids, and glycerol. The strains producing *sn*-1,3-specific lipases which hydrolyze ester bonds at positions *sn*-1 and *sn*-3 of triacylglycerides were found among the bacteria of the genera *Bacillus* and *Pseudomonas*, e.g., *Bacillus megaterium, P. aeruginosa, P. fluorescens* and *Pseudomonas myxogenes* [57]. Lipase C-4 of the bacterium *Bacillus amyloliquefaciens* PS35 has a specificity to long-chain (C_{16}) substrates [58], while the lipase TSLip1 of *T. lipolytica* more effectively hydrolyzes substrates with the short alkyl chains (C_4) [48]. In addition, lipolytic enzymes have various stereospecificity. For example, the esterase of the bacterium *B. megaterium* selectively hydrolyzes racemic mixture of 4-chloro-3-hydroxyethylbutyrate to (*S*)-3-hydroxy-γ-butyrolactone [59].

PRODUCTION OF RECOMBINANT BACTERIAL LIPOLYTIC ENZYMES

Production of industrial enzymes often involves their heterologous expression in well studied strains. These strains can produce proteins that cannot be manufactured in significant quantities with the native strains. It refers to genes of enzymes isolated from metagenomes containing the DNA of uncultured microorganisms. In addition, heterologous expression is used not only for production of native enzymes, but also for bioengineered ones [60].

Both prokaryotic (bacteria) and eukaryotic (yeast, mycelial fungi) hosts are used for heterologous expression [61]. Prokaryotic hosts have a number of disadvantages and are used mainly for laboratory studies. Bacterial strains often cannot provide correct folding, glycosylation, formation of disulfide bonds, and other modifications necessary for functioning of some recombinant proteins [60, 62]. The most frequently used host is *E. coli*, which can produce recombinant proteins with high efficiency (up to 30% of the total cell protein) in an inexpensive cultural media [39]. A major drawback of *E. coli* is its inability for extracellular secretion of recombinant proteins. Fusion of protein with the signal peptide (PelB, OmpA) promotes its secretion into the periplasmic space and its extraction requires the destruction of the cells with ultrasound or with the excessive pressure [60]. It has been shown that the high level of expression of recombinant protein in *E. coli* cells often results in its aggregation and accumulation as the inclusion bodies [63], which prevents its isolation and lowers its yield. *E. coli* is currently used for production of a number of recombinant thermostable bacterial lipases, e.g., from *P. fluorescens* [63], *B. licheniformis* [40], and *Geobacillus stearothermophilus* [18].

One of the approaches to increasing the yield of soluble recombinant proteins of *E. coli* is their co-expression with molecular chaperones. Chaperones are a group of proteins which classification is based on their size, structure, and function. Their main func-

tion is the effective formation of tertiary structures of polypeptide chains in cellular proteins. Chaperones not only promote the correct folding of polypeptide tertiary structures but also participate in proteins [64], refolding of incorrectly folded or aggregated proteins [65], preparation of such aggregates for proteolysis, and induction of apoptosis in damaged cells [66]. It was shown that the co-expression of recombinant proteins and molecular chaperones increases solubility and reduces the aggregation of recombinant proteins in *E. coli*. The correct folding of recombinant proteins requires different systems of chaperones and chaperonin proteins (e.g., GroES, GroEL, ClpB). Chaperones from native enzyme producers can also be used; for example, the co-expression of the lipase of *Pseudomonas cepacia* and the chaperones of this bacterium in *E. coli* produces a highly active enzyme with a specific activity 4850 U/mg and a yield of 314000 mg/g of *E. coli* cells [67]. The co-expression of recombinant enzymes with proteins of the folding system of *E. coli* is often used. For example, the co-expression of lipase Lip-948 of the bacterium *Psychrobacter* sp. and the DnaK-DnaJ-GrpE/ GroES-GroEL chaperone system in *E. coli* BL21(DE3) increases the yield of the soluble protein fraction with the expressed enzyme by 18.8% [68].

BIOTECHNOLOGICAL APPLICATIONS OF LIPOLYTIC ENZYMES AND BIOCATALYSTS

Bacterial lipases and esterases are frequently applied in biotechnology, due to diversity of their properties and catalyzed reactions. In addition, bacterial lipolytic enzymes are usually more resistant to different factors of target processes, e.g., temperature, pH, and the presence of different chemicals in solution [12].

In industry, lipolytic enzymes are used in both the soluble and immobilized forms. Enzyme immobilization produces biocatalysts that can be easily separated from the reaction products and reused [5]. The need to purify reaction products from enzymatic catalysts of the reaction can therefore be avoided. Immobilization often increases the functional thermostability of an enzyme, along with its tolerance to pH and chemical reagents, thereby extending the possibilities of its application [69]. Immobilized bacterial lipolytic enzymes are preferably used in organic synthesis in the food and fuel industries; recently, however, the scope of their application has been greatly extended. Some of the most important industrial applications of lipolytic enzymes are considered below.

Production of Biodiesel Fuel

Fatty acid methyl esters as the main component of biodiesel fuel are preferable produced through the chemical esterification of plant oils with methanol in the presence of homogenous inorganic catalysts (NaOH, KOH and H_2SO_4). Even though chemical esterification ensures high yields of FAMEs, the process is accompanied by high energy consumption (the reaction proceeds at 50–90°C). It is also difficult to separate the catalyst from the reaction products. The glycerol that forms as a side product must be removed, so as the large amounts of wastewater. Enzymatic esterification under mild conditions is therefore an alternative to the conventional processes of biodiesel fuel production [6, 70]. The reactions are performed at low temperatures, including study [71], where the enzymatic esterification of saturated fatty acids with primary aliphatic alcohols (C_4–C_{18}) was carried out at 20–22°C. The reaction of methanolysis is usually conducted with a plant oil/alcohol mixture at a molar ratio of 1 : 3, reflecting the stoichiometry of esterification. The change in this ratio toward higher or lower values usually results in a lower yield of FAMEs [18]. Alternative acyl group acceptors (e.g., methyl acetate and ethyl acetate) are used to prevent the denaturation of enzymes during their interaction with alcohols. It has been shown that when these compounds are used in methanolysis, the yield of FAMEs is 92% at a methyl acetate/oil molar ratio of 12 : 1, with the absence of loss of enzyme activity [72]. The reaction temperature also has a substantial effect on enzyme activity. In contrast to catalytic methanolysis, which proceeds at temperatures below 60°C, the enzymatic methanolysis of oils is most efficient at temperatures below 40°C [73]. At this temperature, protein denaturation is prevented and enzyme activity is retained. The optimum temperature range for the enzymatic esterification of plant oils with methanol is 30–45°C [74]. In addition to plant oils, lipids for esterification can be obtained from microalgae [75, 76]. The authors of [8] described the enzymatic esterification of lipids of the microalga *Micractinium* sp. IC-76 with the *Burkholderia cepacia* lipase immobilized as the cross-linked enzyme aggregates with FAMEs yields of 92.3 ± 1.5%.

Esterification reactions are now performed mainly with the fungal lipase biocatalysts (Novozyme 435, Lipozyme TL IM, Lipozyme RM IM) resulting in FAMEs yields of 90–98% [6]. High yields of FAMEs can also be achieved with immobilized bacterial lipases. In [77], FAMEs were produced using the immobilized (via the inclusion in hydrophobic sol-gel) lipase of the bacterium *P. cepacia*. The optimum reaction conditions were as follows: soybean oil, 10 g; biocatalyst, 475 mg; oil/methanol molar ratio, 1 : 7.5; 35°C; and water, 0.5 g. After 1 h of the reaction, the yield of FAMEs was 67%. The study in [78] was performed with the recombinant lipase of *Staphylococcus haemolyticus* L62, expressed in *E. coli* BL21(DE3) and immobilized on a methacrylic acid–divinylbenzene copolymer through hydrophobic adsorption/inclusion (H-L62 biocatalyst) and hydrophobic adsorption/inclusion with covalent cross-linking (HC-L62 biocatalyst). Both biocatalysts proved to be stable at

high temperatures and in the broad pH range; however, the HC-L62 biocatalyst was more effective in thereuse.

For the biodiesel fuel production, it is highly important to use lipases tolerant not only to high temperatures but also to high concentrations of organic solvents. The study performed in [79] with the lipase of the bacterium *Acinetobacter baylyi* (ABL) immobilized on Sepabeads EC-OD showed that immobilization resulted in the increased pH and storage stability of the enzyme. The optimum conditions for esterification of palm oil with methanol for 24 h at 40°C were as follows: oil/methanol molar ratio, 1 : 4; 20% (w/v) biocatalyst, 4% water. Under these conditions, the yield of FAMEs was 93%. Lipase of the bacterium *Bacillus aerius* immobilized on silica gel showed high operational stability during the esterification at high temperatures. When the castor oil and methanol were used at a molar ratio of 1 : 4 at 55°C for 96 h, the yield of FAMEs for this enzyme was 78.13% [80]. The high yield of FAMEs (100%) was achieved in the reaction of esterification of olive oil with methanol at a ratio of 1 : 3 at 35°C for 18 h using recombinant lipase LipBA from *B. amyloliquefaciens* immobilized on sodium alginate and expressed in *E. coli* BL21(DE3) with the pET-28a plasmid [81].

In addition of the high yields of reaction products, application of immobilized enzymes for esterification allows the biocatalysts to be easily separated from reaction products and reused. In the study [82], *B. cepacia* lipase immobilized in *n*-butyl-substituted hydrophobic silica monoxide retained about 80% of the initial activity during 49 days of continuous esterification at 40°C of jatropha seed oil with methanol at a molar ratio of 1 : 3 and 0.6% water. In another work, the same lipase was immobilized through cross-linking with glutaraldehyde followed by inclusion in a hybrid matrix consisting of the alginate/κ-carrageenan mixture. The biocatalyst was used for esterification of jatropha seed oil under the following conditions: oil, 10 g; oil/methanol molar ratio, 1 : 10; water, 1 g; immobilized lipase, 5.25 g. The yield of FAMEs under these conditions was close to 100%, and the biocatalyst retained 73% of its activity after six cycles of operation [83]. The high operating stability of the *B. cepacia* lipase was also shown after its immobilization on aminated magnetic nanoparticles. Under the optimal conditions, the yield of FAMEs was 96.8% after 12 h and the biocatalyst retained more than 60% of its activity after 15 reaction cycles [84].

In addition to the lipase of *B. cepacia*, the properties of immobilized thermostable lipases produced by bacteria of the genus *Geobacillus* have been studied. The authors of [85] achieved 62–76% immobilization of the recombinant lipase of *Geobacillus thermocatenulatus* (BTL2) on glyoxyl agarose using dithiothreitol as an additive. The resulting biocatalyst was active at a high temperature (70°C) with a half-inactivation time of 54.5 h and was applied for esterification of cod liver oil. The lipase of strain *G. stearothermophilus* G3 immobilized on silica gel was used as a biocatalyst for sunflower oil methanolysis (methanol/oil molar ratio of 3 : 1) with4% (w/v) water at 40°C [18]. The yield of FAMEs under these conditions was 40–43% after 96 h.

Due to the high activity and functional stability in the presence of alcohols and other organic solvents, lipolytic enzymes of bacterial origin are used in biodiesel fuel production. Biocatalysts based on bacterial lipases demonstrate high performance characteristics and high yields of FAMEs.

Production of Aromatic Compounds

Application of bacterial lipolytic enzymes in food industry is limited mainly to the production of esters and alcohols as flavoring agents. Lipase of *Lactobacillus plantarum*, immobilized in a 3% sodium alginate suspension, catalyzes the synthesis of short-chain fatty acid esters including hydroxybenzyl acetate and triazole ester derivatives (yields of 70–78.7% and 16.66%, respectively) [86]. The resulting product (4-hydroxybenzyl acetate) is also used in cosmetic industry as sunblock. Another bacterial lipase, isolated from *Staphylococcus simulans* and adsorbed on $CaCO_3$, was used as a biocatalyst for the production of ethyl valerate and hexyl acetate. These short-chain esters with fruit flavor are used in the food, cosmetics, and pharmaceutical industries [87]. Reaction between valeric acid and ethanol at a molar ratio of 1 : 1, with the addition of 20% (w/v) water and 200 U of immobilized lipase produced ethyl valerate at a yield of 51% at 37 °C. The biocatalyst activity remained unchanged over 10 reaction cycles. The reaction between acetic acid and hexanol (molar ratio, 1 : 1) at 37°C, with the addition of 10% (w/v) water and 100 U of immobilized lipase, resulted in an hexyl acetate yield of 41%. The biocatalyst remained stable over five reaction cycles.

L-menthol is a cyclic monoterpene alcohol and one of the most widely used components of drugs and flavoring agents in tobacco and confectionery products and is mainly produced from natural sources (peppermint oil). Since the production of natural menthol cannot fully satisfy needs of the food and pharmaceutical industries the chemical and biological synthesis is also used for its manufacturing. The cross-linked aggregates of recombinant esterase BsE from the bacterium *Bacillus subtilis* 0554 were applied as a biocatalyst for (*D*,*L*)-menthyl acetate hydrolysis for *L*-menthol production with >40% conversion and an enantiomeric excess $ee_p > 97\%$ [88].

Production of chemicals used in the food and pharmaceutical industries requires the development of new ecologically pure technologies that have no negative effects on human health. The production of different aromatic substances through biocatalytic synthesis is therefore a promising approach due to high enzyme

activity and specificity, mild reaction conditions, and the high purity of products.

Organic Synthesis

Bacterial esterases are widely used in organic synthesis, due to their ability to provide stereo- and regioselective catalysis [20] in addition to their high resistance to organic solvents. The resulting products with a high purity, are used in the chemical synthesis of pharmaceutical substances.

Carboxylesterase NP produced by *B. subtilis*, is one of the best-studied bacterial enzymes used for the synthesis of optically pure organic chemicals. This enzyme is used for the enantioselective hydrolysis of derivatives of 2-arylpropionic acid, including nonsteroidal anti-inflammatory drugs naproxen and ibuprofen, which *R*- and *S*-enantiomers with substantially different pharmacological activities. The carboxylesterase NP from genus *Bacillus* originally can be effectively applied for selective separation of enantiomers of (*R,S*)-2-(6-methoxy-2-naphthyl)propionic acid methyl ester (naproxen methyl ester), with a 95% yield of its *S*-enantiomer with high optical purity (enantiomeric excess $ee_p = 99\%$) [89]. Enzyme inactivation as a result of interaction between carbonic acid products and the amino groups of essential amino acids (e.g., lysine) [90], is prevented by preliminary modification of carboxylesterase with chemical reagents. It was shown that carboxylesterase NP pretreated with formaldehyde at a concentration of 2300 ppm can be used for the laboratory-scale production of naproxen *S*-enantiomer [91].

Ketoprofen (2-(3-benzoylphenyl)propionic acid) is the widely used drug and is synthesized an a mixture of two enantiomers. It has been shown that the *S*-enantiomer of ketoprofen is more pharmacologically active. A mixture of these enantiomers was separated in [17] using a recombinant esterase of *Pseudomonas* sp. KCTC 10122BP, with a 99% enantiomeric excess of the *S*-form during hydrolysis.

In addition to the synthesis of substances used as nonsteroidal anti-inflammatory drugs, bacterial lipolytic enzymes are successfully applied in other reactions. Recombinant esterase ybfF, isolated from *E. coli* K-12 PA340/T6 and expressed with pET26b plasmid in *E. coli* BL21(DE3), exhibits high enantioselectivity in the hydrolysis of (*R,S*)-1-phenyl ethyl acetate resulting in enantiomeric excess $ee_p > 99\%$ of (*R*)-1-phenylethanol [92]. This enzyme also shows high selectivity with respect to butyl and capryl isopropylidene glycerol esters with enantiomeric excesses of *R*-enantiomer of 72 to 94%.

The enantioselectivity of enzymes can be improved in several ways, including enzyme immobilization and addition of surfactants, ionic liquids and organic solvents to reaction mixture. For example, ibuprofen (another derivative of propionic acid) was produced in the presence of ionic liquids using thermostable esterase EST10 from the bacterium *Thermotoga maritima* [93]. The *S*-enantiomer which was produced after hydrolysis of (*R,S*)-2-(4-isobutylphenyl)propionic acid ethyl esters in the presence of [OmPy][BF_4], had $ee_p = 96.6\%$, enantiomeric ratio $E = 177.0$. The enantioselectivity of thermostable bacterial esterase EST10 was higher than the immobilized lipase from *Candida rugosa* ($E = 19$; $ee_p = 83\%$) [94] and the time of reaction reduced from 24 to 10 h. In [95], the lipase of the bacterium *B. cepacia* G63, immobilized on ion-exchange resin, was used to separate a racemic mixture of ketoprofen ethyl esters with enantiomeric ratio $E = 10.01$.

Bioengineering is widely used to improve the enantioselectivity of enzymes include substituting amino acids in protein via random and site-directed mutagenesis. In [96], the enantioselectivity of esterase Est25 was enhanced by substituting amino acid L255W ($E = 18$) as compared with initial esterase ($E = 1$) for resolution of a racemic mixture of ketoprofen ethyl esters. At the same time, adding polar solvent as methanol (20% (v/v)) and ethanol (4–20% (v/v)) to the reaction mixture increased the enantioselectivity of mutant esterase Est25 by 10 times ($E > 200$). Enantioselectivity of enzymes can be enhanced through mutagenesis resulting in two, three, or more amino acid substitutions. Two amino acid substitutions that affect enzyme specificity, V138G ($E = 3.0 \pm 0.1$) and L200R ($E = 4.8 \pm 0.1$), were identified for the Est-AF esterase isolated from *Archaeoglobus fulgidus* DSM 4304 (enantioselectivity of the initial esterase was $E = 0.7 \pm 0.0$). The enzyme with both substitutions (V138G/L200R) had high level of enantioselectivity: $E = 19.5 \pm 0.5$. Addition of various surfactants (1% (w/v) EDTA, Triton X-100, Tween 80) and organic solvents (20% (v/v) *n*-hexane, methanol) to the reaction medium resulted in a higher enantiomeric ratio: $E = (21.8 \pm 0.1)–(37.7 \pm 0.3)$ [97].

The enantioselectivity of enzymes can be increased by random mutagenesis. In [98], it was shown that application of the UV mutant of *Trichosporon brassicae* CGMCC 0574 in the separation of ketoprofen ethyl esters resulted in enantiomeric ratio increase up to 21.1–31.0, while the unmodified strain had no appreciable enantioselective activity.

Application of bacterial lipases and esterases in the production of optically pure compounds is one of the most important industrial applications of enzymes. These enzymes have a diverse substrate specificity with the possibility of their modification, in this respect allowing to use them for production of high purity products.

Biodegradation of Pesticides in Wastewater

In addition to the conventional ways of pesticide detoxification (mineralization) in wastewaters (e.g., chemical oxidation [99], ultraviolet radiation [100]

and nanofiltration [99]) the biological treatment with bacteria [101], fungi [102], and algae [103] are applied. Biological treatment has recently become preferable approach due to its relatively low cost and labor intensity; in addition, biological treatment in most cases provides the complete hydrolysis of pesticides. Pesticides such as malathion [102], pendimethalin [104], profenofos [105], parathion [106], cyhalothrin [107] in aqueous solutions are usually biodegraded by bacterial strains of the genera *Bacillus*, *Pseudomonas*, *Acinetobacter* and *Serratia* [101, 107]. These bacteria produce lipolytic enzymes that hydrolyze the ester bonds in molecules of pesticides. The biodegradation of malathion in aqueous solutions with the participation of lipolytic bacteria of the genus *Bacillus* takes from several hours to several days. For example, the strain *B. licheniformis* ML-1 ensures 78% removal of malathion from aqueous solutions over 5 days at 32 °C and pH 7.5, and an initial malathion concentration of 25 mg/L [108]. It was shown that the efficiency of pesticides removal increases with the immobilization of bacteria [105]; however, the degree and rate of pesticide hydrolysis remain insufficient.

Application of purified bacterial enzymes increases the degree of malathion removal. Carboxylesterase D1CarE5 from *A. tengchongensis* was cloned in the pET28(+) vector and expressed in *E. coli* BL21(DE3) can effectively hydrolyze malathion (by 89%) in a 25 mM phosphate buffer at pH 7.0 over 100 min [27]. The authors [21] studied the application of biocatalyst based on cross-linked aggregates (CLEA-estUT1) of recombinant thermostable esterase estUT1 of the bacterium *Ureibacillus thermosphaericus* for malathion degradation [109]. The possibility of directly application of the lipolytic enzyme-based biocatalyst for malathion removal from wastewater was demonstrated for the first time. The degree of malathion removal was 99.5 ± 1.4% after 14 h of the reaction at 37°C, with an initial malathion concentration of 27.5 mg/L. CLEA-estUT1 catalyst remained stable in the reaction of malathion hydrolysis: the extent of malathion removal (25 cycle) was 55.2 ± 1.1%.

Production of Detergents

One of applications of bacterial lipases is enhancement of synthetic detergents effectiveness against fat stains when used in combination with amylases and proteases [19]. Due to the diverse properties of bacterial lipases (thermostability, functional stability at alkaline pH, water solubility, and resistance to surfactants and proteases), they can be used as components of both liquid and solid detergents [110]. Application of the cold-active enzymes reduces the temperature and energy consumption during the stain removal [111]. Commercial bacterial lipase Lipomax (*Pseudomonas alcaligenes*) and Lumafast (*Pseudomonas mendocina*) are aimed to be used in detergent formulations [44]. Stable lipases can also be found in bacteria of the genera *Bacillus* and *Burkholderia* [112]. In [113], alkaline lipase of bacterium *B. cepacia* RGP-10 exhibited high resistance to ionic and nonionic surfactants, and to commercial detergents. This lipase retained 100% of its activity in the presence of strong oxidants as H_2O_2, sodium perborate, and sodium hypochlorite after 1 h of treatment at a room temperature. The enzyme was also stable in the presence of commercial proteases, retaining 100% of its activity after 1 h of incubation at 50°C.

Bacterial lipases can effectively clean fabrics without detergents. The authors of [114] reported the immobilization of lipase from the bacterium *P. fluoresces* on woolen fabric according to a protocol that included fabric chlorination, polyethylenimine adsorption, and adding glutaraldehyde, followed by enzyme adsorption. Immobilized lipase proved to be effective for removing of oil stains, which were easily eliminated from the fabric without detergents after 24 h of storage at room temperature. It was noted that the lipase immobilized on the fabric remained stable up to 80 days when stored at lower temperatures. Enzyme immobilization on fabrics is thus a step toward making fabrics with improved cleaning properties.

CONCLUSIONS

Analysis of properties and applications of hydrolytic enzymes is an important biotechnological task. The diverse properties of bacterial lipases and esterases (broad substrate specificity, stability at high temperatures and different pH, resistance to metal cations and organic solvents) reveal their prospective application in various biotechnological processes. Current studies are therefore aimed at screening, isolating, and study of properties new bacterial lipolytic enzymes; bioengineering of enzymes with improved activity and selectivity; and developing immobilization techniques in order to obtain more active and stable biocatalysts.

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REFERENCES

1. Sorokina, K.N., Samoylova, Y.V., Piligaev, A.V., Sivakumar, U., and Parmon, V.N., *Catal. Ind.,* 2017, vol. 9, no. 3, pp. 264–269.
2. Sorokina, K.N., Samoylova, Y.V., Piligaev, A.V., Sivakumar, U., and Parmon, V.N., *Catal. Ind.,* 2017, vol. 9, no. 4, pp. 348–353.
3. Samoylova, Y.V., Sorokina, K.N., and Parmon, V.N., *Catal. Ind.,* 2016, vol. 9, no. 1, pp. 62–70.
4. Samoylova, Y.V., Piligaev, A.V., Sorokina, K.N., and Parmon, V.N., *Catal. Ind.,* 2017, vol. 9, no. 1, pp. 62–70.

5. Gotovtsev, P.M., Yuzbasheva, E.Yu., Gorin, K.V., Butylin, V.V., Badranova, G.U., Perkovskaya, N.I., Mostova, E.B., Namsaraev, Z.B., Rudneva, N.I., Komova, A.V., Vasilov, R.G., and Sineokii, S.P., *Biotekhnol.,* 2015, no. 2, pp. 33–45.

6. Yuzbasheva, E.Yu., Gotovtsev, P.M., Mostova, E.B., Perkovskaya, N.I., Lomonosova, M.A., Butylin, V.V., Sineokii, S.P., and Vasilov, R.G., *Biotekhnol.,* 2014, no. 1, pp. 8–24.

7. Sarmah, N., Revathi, D., Sheelu, G., Yamuna Rani, K., Sridhar, S., Mehtab, V., and Sumana, C., *Biotechnol. Prog.,* 2018, vol. 34, no. 1, pp. 5–28.

8. Sorokina, K.N., Samoilova, Yu.V., Piligaev, A.V., Tulupov, A.A., and Parmon, V.N., *Primenenie biotekhnologii dlya pererabotki lipidov rastitel'nogo proiskhozhdeniya v tsennye produkty i ikh vliyanie na zdorov'e cheloveka* (Application of Biotechnology for the Conversion of Plant-Derived Lipids into Valuable Products and Their Effect on Human Health), Novosibirsk: Novosib. Gos. Univ., 2017.

9. Khan, F.I., Lan, D., Durrani, R., Huan, W., Zhao, Z., and Wang, Y., *Front. Bioeng. Biotechnol.,* 2017, vol. 5, p. 16. https://doi.org/10.3389/fbioe.2017.00016

10. Hide, W.A., Chan, L., and Li, W.H., *J. Lipid Res.,* 1992, vol. 33, no. 2, pp. 167–178.

11. Kapoor, M. and Gupta, M.N., *Proc. Biochem.,* 2012, vol. 47, no. 4, pp. 555–569.

12. Bornscheuer, U.T., *FEMS Microbiol. Rev.,* 2002, vol. 26, no. 1, pp. 73–81.

13. Lee, L.-C., Lee, Y.-L., Leu, R.-J., and Shaw, J.-F., *Biochem. J.,* 2006, vol. 397, no. 1, pp. 69–76.

14. Bharathi, D., Rajalakshmi, G., and Komathi, S., *J. King Saud Univ., Sci.,* 2018. https://doi.org/10.1016/j.jksus.2017.12.018

15. Samoilova, Yu.V., Tulupov, A.A., and Sorokina, K.N., *Vestn. Novosib. Gos. Univ., Ser. Biol. Klin. Med.,* 2014, vol. 12, no. 4, pp. 77–84.

16. Sorokina, K.N., Rozanov, A.S., Bryanskaya, A.V., and Pel'tek, S.E., *Vavilov. Zh. Genet. Sel.,* 2013, vol. 17, nos. 4/1, pp. 651–658.

17. Kim, G.J., Lee, E.G., Gokul, B., Hahm, M.S., Prerna, D., Choi, G.S., Ryu, Y.W., Ro, H.-S., and Chung, B.H., *J. Mol. Catal. B: Enzym.,* 2003, vol. 22, no. 1, pp. 29–35.

18. Samoylova, Y.V., Piligaev, A.V., Sorokina, K.N., Rozanov, A.S., Peltek, S.E., Novikov, A.A., Almyasheva, N.R., and Parmon, V.N., *Catal. Ind.,* 2016, vol. 8, no. 2, pp. 187–193.

19. Nagibina, V.V., Rebezov, M.B., Anokhina, E.S., Maksimyuk, N.N., and Asenova, B.K., *Molodoi Uchenyi.,* 2014, no. 8, pp. 214–216.

20. Bezborodov, A.M. and Zagustina, N.A., *Appl. Biochem. Microbiol.,* 2014, vol. 50, no. 4, pp. 313–337.

21. Samoylova, Y.V., Sorokina, K.N., Piligaev, A.V., and Parmon, V.N., *Catalysts,* 2018, vol. 8, no. 4, p. 154.

22. Bayoumi, R.A., El-Louboudey, S.S., Sidkey, N.M., and Abd-El-Rahman, M.A., J. Appl. Sci. Res. (*Ma'an, Jordan*), 2007., vol. 3, no. 12, pp. 1752–1765.

23. Chiş, L., Hriscu, M., Bica, A., Toşa, M., Nagy, G., Róna, G., Vértessy, B.G., and Irimie, F.D., *J. Gen. Appl. Microbiol.,* 2013, vol. 59, no. 2, pp. 119–134.

24. Kim, J., Deng, L., Hong, E., and Ryu, Y., *Protein Expression Purif.,* 2015, vol. 116, pp. 90–97.

25. Shariff, F.M. and Rahman, R.N.Z.R.A., Basri, M., and Salleh, A.B., *Int. J. Mol. Sci.,* 2011, vol. 12, no. 5, pp. 2917–2934.

26. Sivaramakrishnan, R. and Muthukumar, K., *Appl. Biochem. Biotechnol.,* 2012, vol. 166, no. 4, pp. 1095–1111.

27. Xie, Z., Xu, B., Ding, J., Liu, L., Zhang, X., Li, J., and Huang, Z., *Biotechnol. Lett.,* 2013, vol. 35, no. 8, pp. 1283–1289.

28. Mohamed, Z.K., Ahmed, M.A., Fetyan, N.A., and Elnagdy, S.M., *J. Adv. Res.,* 2010, vol. 1, no. 2, pp. 145–149.

29. Surendhiran, D., Sirajunnisa, A.R., and Vijay, M., *3 Biotech,* 2015, vol. 5, no. 5, pp. 715–725.

30. Leow, T.C., Sharif, F.M., Rahman, R.N.Z.R.A., Salleh, A.B., and Basri, M., in *Book of Thermostable Lipases,* New York: Nova Science Publishers, 2006, pp. 41-61.

31. Vieille, C. and Zeikus, G.J., *Microbiol. Mol. Biol. Rev.,* 2001, vol. 65, no. 1, pp. 1–43.

32. Castro-Ochoa, L.D., Rodríguez-Gómez, C., Valerio-Alfaro, G., and Oliart Ros, R., *Enzyme Microb. Technol.,* 2005, vol. 37, no. 6, pp. 648–654.

33. Stathopoulou, P.M., Savvides, A.L., Karagouni, A.D., and Hatzinikolaou, D.G., *BioMed Res. Int.,* 2013. https://doi.org/10.1155/2013/703130

34. Salameh, M.A. and Wiegel, J., *Appl. Environ. Microbiol.,* 2007, vol. 73, no. 23, pp. 7725–7731.

35. Kriger, A.V., Dyshlyuk, L.S., Dolganyuk, V.F., Zimina, M.I., and Asyakina, L.K., *Fundam. Issled.: Khim. Nauki,* 2013, no. 12, pp. 122–126.

36. Sorokina, K.N., Nuriddinov, M.A., Rozanov, A.S., Ivanisenko, V.A., and Pel'tek, S.E., *Vavilovsk. Zh. Genet. Sel.,* 2013, vol. 17, no. 4/1, pp. 666–674.

37. Litthauer, D., Ginster, A., van Eeden Skein, E., *Enzyme Microb. Technol.,* 2002, vol. 30, no. 2, pp. 209–215.

38. Nawani, N. and Kaur, J., *Mol. Cell. Biochem.,* 2000, vol. 206, no. 1, pp. 91–96.

39. Gricajeva, A., Bendikienė, V., and Kalėdienė, L., *Int. J. Biol. Macromol.,* 2016, vol. 92, pp. 96–104.

40. Kaur, G., Singh, A., Sharma, R., Sharma, V., Verma, S., and Sharma, P.K., *3 Biotech,* 2016, vol. 6, no. 1, p. 4

41. Bradoo, S., Saxena, R.K., and Gupta, R., *World J. Microbiol. Biotechnol.,* 1999, vol. 15, no. 1, pp. 87–91.

42. Ramani, K., Chockalingam, E., and Sekaran, G., *J. Ind. Microbiol. Biotechnol.,* 2010, vol. 37, no. 5, pp. 531–535.

43. Andersson, R.E., Hedlund, C.B., and Jonsson, U., *J. Dairy Sci.,* 1979, vol. 62, no. 3, pp. 361–367.

44. Sharma, S. and Kanwar, S.S., *Sci. World J.,* 2014, vol. 2014. https://doi.org/10.1155/2014/625258

45. Schmidt-Dannert, C., Sztajer, H., Stöcklein, W., Menge, U., Schmid, R.D., *Biochim. Biophys. Acta, Lipids Lipid Metab.,* 1994, vol. 1214, no. 1, pp. 43–53.

46. Sugihara, A., Tani, T., and Tominaga, Y., *J. Biochem.,* 1991, vol. 109, no. 2, pp. 211–216.
47. Hun, C.J., Rahman, R.N.Z.A., Salleh, A.B., and Basri, M., *Biochem. Eng. J.,* 2003, vol. 15, no. 2, pp. 147–151.
48. Gumerov, V.M., Mardanov, A.V., Kolosov, P.M., and Ravin, N.V., *Appl. Biochem. Microbiol.,* 2012, vol. 48, no. 4, pp. 338–343.
49. Macrae, A.R. and Hammond, R.C., *Biotechnol. Genet. Eng. Rev.,* 1985, vol. 3, no. 1, pp. 193–218.
50. Balan, A., Ibrahim, D., Abdul Rahim, R., and Ahmad Rashid, F.A., *Enzyme Res.,* 2012, vol. 2012. https://doi.org/10.1155/2012/987523
51. Bisht, D., Yadav, S.K., and Darmwal, N.S., *Braz. J. Microbiol.,* 2013, vol. 44, no. 4, pp. 1305–1314.
52. Ekinci, A.P., Dinçer, B., Baltaş, N., and Adıgüzel, A., *J. Enzyme Inhib. Med. Chem.,* 2016, vol. 31, no. 2, pp. 325–331.
53. Lestari, P., Raharjo, T.J., Matsjeh, S., and Haryadi, W., *AIP Conf. Proc.,* 2016, vol. 1755, no. 1, p. 080003.
54. Dharmsthiti, S., Pratuangdejkul, J., Theeragool, G., and Luchai, S., *J. Gen. Appl. Microbiol.,* 1998, vol. 44, no. 2, pp. 139–145.
55. Jaeger, K.-E., Ransac, S., Dijkstra, B.W., Colson, C., van Heuvel, M., and Misset, O., *FEMS Microbiol. Rev.,* 1994, vol. 15, no. 1, pp. 29–63.
56. Hassing, G.S., *Biochim. Biophys. Acta, Enzymol.,* 1971, vol. 242, no. 2, pp. 381–394.
57. Lanser, A.C., Manthey, L.K., and Hou, C.T., *Curr. Microbiol.,* 2002, vol. 44, no. 5, pp. 336–340.
58. Kanmani, P., Kumaresan, K., and Aravind, J., *Braz. J. Microbiol.,* 2015, vol. 46, no. 4, pp. 1235–1243.
59. Zheng J.-Y., Wang J., Zhou S.-S., Li X.-J., Ying X.-X., and Wang, Z., *Protein Expression Purif.,* 2017, vol. 136, pp. 66–72.
60. Valero, F., in *Lipases and Phospholipases: Methods and Protocols,* Sandoval, G., Ed., Totowa, NJ: Humana Press, 2012, pp. 161–178.
61. Borrelli, G.M. and Trono, D., *Int. J. Mol. Sci.,* 2015, vol. 16, no. 9, pp. 20774–20840.
62. Padkina, M.V. and Sambuk, E.V., *Ekol. Genet.,* 2015, vol. 13, no. 2, pp. 36–57.
63. Villaverde, A. and Mar Carrió, M., *Biotechnol. Lett.,* 2003, vol. 25, no. 17, pp. 1385–1395.
64. De Marco, A., *Microb. Cell Fact.,* 2009, vol. 8, no. 1, p. 26.
65. De Marco, A., Deuerling, E., Mogk, A., Tomoyasu, T., and Bukau, B., *BMC Biotechnol.,* 2007, vol. 7, p. 32.
66. Saibil, H.R., *Curr. Opin. Struct. Biol.,* 2008, vol. 18, no. 1, pp. 35–42.
67. Quyen, D.T., Schmidt-Dannert, C., and Schmid, R.D., *Appl. Environ. Microbiol.,* 1999, vol. 65, no. 2, pp. 787–794.
68. Cui, S.-S., Lin, X.-Z., and Shen, J.-H., *Protein Expression Purif.,* 2011, vol. 77, no. 2, pp. 166–172.
69. Datta, S., Christena, L.R., and Rajaram, Y.R.S., *3 Biotech,* 2013, vol. 3, no. 1, pp. 1–9.
70. Kovalenko, G.A., Perminova, L.V., Beklemishev, A.B., Mamaev, A.L., and Patrushev, Yu.V., *Catal. Ind.,* 2018, vol. 10, no. 1, pp. 68–74.
71. Kovalenko, G.A., Perminova, L.V., Beklemishev, A.B., and Pykhtina, M.B., *Vestn. Permsk. Nats. Issled. Politekh. Univ. Khim. Tekhnol. Biotechnol.,* 2018, no. 2, pp. 7–22.
72. Du, W., Xu, Y., Liu, D., and Zeng, J., *J. Mol. Catal. B: Enzym.,* 2004, vol. 30, no. 3, pp. 125–129.
73. Gonzalo, A., García, M., Luis Sánchez, J., Arauzo, J., and Peña, J.Á., *Ind. Eng. Chem. Res.,* 2010, vol. 49, no. 9, pp. 4436–4443.
74. Salis, A., Pinna, M., Monduzzi, M., and Solinas, V., *J. Biotechnol.,* 2005, vol. 119, no. 3, pp. 291–299.
75. Sorokina, K.N., Yakovlev, V.A., Piligaev, A.V., Kukushkin, R.G., Pel'tek, S.E., Kolchanov, N.A., and Parmon, V.N., *Catal. Ind.,* 2012, vol. 4, no. 3, pp. 202–208.
76. Piligaev, A.V., Sorokina, K.N., and Parmon, V.N., *Vestn. Novosib. Gos. Univ., Ser. Biol. Klin. Med.,* 2015, vol. 13, no. 4, pp. 19–26.
77. Noureddini, H., Gao, X., and Philkana, R.S., *Bioresour. Technol.,* 2005, vol. 96, no. 7, pp. 769–777.
78. Kim, S.H., Kim, S.-J., Park, S., and Kim, H.K., *J. Mol. Catal. B: Enzym.,* 2013, vols. 85–86, pp. 10–16.
79. Winayanuwattikuna, P., Piriyakananona, K., Wongsathonkittikunb, P., and Charoenpanich, J., *ScienceAsia,* 2014, vol. 40, no. 5, pp. 327–334.
80. Narwal, S.K., Saun, N.K., Dogra, P., Chauhan, G., and Gupta, R., *BioMed Res. Int.,* 2015, vol. 2015. https://doi.org/10.1155/2015/281934
81. Cai, X., Ma, J., Wei, D.-Z., Lin, J.-P., Wei, W., *Antonie van Leeuwenhoek,* 2014, vol. 106, no. 5, pp. 1049–1060.
82. Kawakami, K., Oda, Y., and Takahashi, R., *Biotechnol. Biofuels,* 2011, vol. 4, p. 42.
83. Abdulla, R. and Ravindra, P., *Biomass Bioenergy,* 2013, vol. 56, pp. 8–13.
84. Li, K., Fan, Y., He, Y., Zeng, L., Han, X., and Yan, Y., *Sci. Rep.,* 2017, vol. 7, no. 1. https://www.nature.com/articles/s41598-017-16626-5. Cited April 8, 2019.
85. Godoy, C.A., *Int. J. Mol. Sci.,* 2017, vol. 18, no. 10, p. 2130.
86. Uppada, S.R., Akula, M., Bhattacharya, A., and Dutta, J.R., *J. Genet. Eng. Biotechnol.,* 2017, vol. 15, no. 2, pp. 331–334.
87. Karra-Châabouni, M., Ghamgui, H., Bezzine, S., Rekik, A., and Gargouri, Y., *Process Biochem.,* 2006, vol. 41, no. 7, pp. 1692–1698.
88. Pan, J., Dang, N.-D., Zheng, G.-W., Cheng, B., Ye, Q., and Xu, J.-H., *Bioresour. Bioprocess.,* 2014, vol. 1, p. 12. https://doi.org/10.1186/s40643-014-0012-x
89. Quax, W.J. and Broekhuizen, C.P., *Appl. Microbiol. Biotechnol.,* 1994, vol. 41, no. 4, pp. 425–431.
90. Mutsaers, J.H.G.M. and Kooreman, H.J., *Recl. Trav. Chim. Pays-Bas,* 1991, vol. 110, no. 5, pp. 185–188.
91. Steenkamp, L. and Brady, D., *Process Biochem.,* 2008, vol. 43, no. 12, pp. 1419–1426.
92. Godinho, L.F., Reis, C.R., Tepper, P.G., Poelarends, G.J., and Quax, W.J., *Appl. Environ. Microbiol.,* 2011, vol. 77, no. 17, pp. 6094–6099.
93. Wei, T., Yang, K., Bai, B., Zang, J., Yu, X., and Mao, D., *Molecules,* 2016, vol. 21, no. 7, p. 905. https://doi.org/10.3390/molecules21070905

94. Marszałł, M.P. and Siódmiak, T., *Catal. Commun.,* 2012, vol. 24, pp. 80–84.
95. Li, X., Liu, T., Xu, L., Gui, X., Su, F., and Yan, Y., *Biotechnol. Bioprocess Eng.,* 2012, vol. 17, no. 6, pp. 1147–1155.
96. Yoon, S., Kim, S., Park, S., Hong, E., Kim, J., Kim, S., Yoo, T.H., and Ryu, Y., *J. Mol. Catal. B: Enzym.,* 2014, vol. 100, pp. 25–31.
97. Kim, J., Kim, S., Yoon, S., Hong, E., and Ryu, Y., *Appl. Microbiol. Biotechnol.,* 2015, vol. 99, no. 15, pp. 6293–6301.
98. Wu, H.-Y., Xu, J.-H., Shen, D., and Xin, Q., *J. Ind. Microbiol. Biotechnol.,* 2003, vol. 30, no. 6, pp. 357–361.
99. Zhang, Y. and Pagilla, K., *Desalination,* 2010, vol. 263, nos. 1–3, pp. 36–44.
100. Shayeghi, M., Dehghani, M.H., Alimohammadi, M., and Goodini, K., *Iran. J. Arthropod Borne Dis.,* 2012, vol. 6, no. 1, pp. 45–53.
101. Singh, B., Kaur, J., and Singh, K., *World J. Microbiol. Biotechnol.,* 2012, vol. 28, no. 3, pp. 1133–1141.
102. Kim, Y.-H., Ahn, J.-Y., Moon, S.-H., and Lee, J., *Chemosphere,* 2005, vol. 60, no. 10, pp. 1349–1355.
103. Ibrahim, W.M., Karam, M.A., El-Shahat, R.M., and Adway, A.A., *BioMed Res. Int.,* 2014, vol. 2014. https://doi.org/10.1155/2014/392682
104. More, V.S., Tallur, P.N., Niyonzima, F.N., and More, S.S., *3 Biotech,* 2015, vol. 5, no. 6, pp. 967–974.
105. Talwar, M.P. and Ninnekar, H.Z., *J. Basic Microbiol.,* 2015, vol. 55, no. 9, pp. 1094–1103.
106. Pailan, S., Gupta, D., Apte, S., Krishnamurthi, S., and Saha, P., *Int. Biodeterior. Biodegrad.,* 2015, vol. 103, pp. 191–195.
107. Chen, S., Deng, Y., Chang, C., Lee, J., Cheng, Y., Cui, Z., Zhou, J., He, F., Hu, M., and Zhang, L.-H., *Sci. Rep.,* 2015, vol. 5. https://www.nature.com/articles/srep08784. Cited April 8, 2019.
108. Khan, S., Zaffar, H., Irshad, U., Ahmad, R., Khan, A.R., Shah, M.M., Bilal, M., Iqbal, M., and Naqvi, T., *Arch. Biol. Sci.,* 2016, vol. 68, no. 1, pp. 51–59.
109. Samoylova, Y.V., Sorokina, K.N., Romanenko, M.V., and Parmon, V.N., *Extremophiles,* 2018, vol. 22, no. 2, pp. 271–285.
110. Chauhan, M., Chauhan, R.S., and Garlapati, V.K., *BioMed Res. Int.,* 2013, vol. 2013. https://doi.org/10.1155/2013/374967
111. Joseph, B., Ramteke, P.W., and Thomas, G., *Biotechnol. Adv.,* 2008, vol. 26, no. 5, pp. 457–470.
112. Kanjanavas, P., Khuchareontaworn, S., Khawsak, P., Pakpitcharoen, A., Pothivejkul, K., Santiwatanakul, S., Matsui, K., Kajiwara, T., and Chansiri, K., *Int. J. Mol. Sci.,* 2010, vol. 11, no. 10, pp. 3783–3792.
113. Rathi, P., Saxena, R.K., and Gupta, R., *Process Biochem.,* 2001, vol. 37, no. 2, pp. 187–192.
114. An, J.D., Patterson, D.A., McNeil, S., and Hossain, M.M., *Biotechnol. Prog.,* 2014, vol. 30, no. 4, pp. 806–817.

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