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Review

Lipases from the genus *Penicillium*: Production, purification, characterization and applications

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ABSTRACT

Lipases are ubiquitous enzymes with considerable industrial potential since they have not only general advantages of biocatalysts, such as high catalytic activity, mild reaction conditions, environmental friendliness, and exquisite chemical, enantio- and regioselectivity, but also very broad substrate range and excellent stability. A number of *Penicillia* are good producers of lipases; some of them are already commercialized. In the past two decades, the lipases from the genus *Penicillium* have been studied extensively by the researchers. In this review, we presented an overview on their production, purification, biochemical properties, specificity, tolerance toward surfactants, inhibitors and organic solvents, and applications.

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Abbreviations: AOT, sodium di(2-ethylhexyl) sulfosuccinate (Aerosol OT); CLA, conjugated linoleic acid; COD, chemical oxygen demand; DAG, diacylglycerol; MAG, monoacylglycerol; MDGL, mono- and di-acylglycerols lipase; OMW, olive mill wastewater; PCR, polymerase chain reaction; SEP, solid enzyme preparation; SmF, submerged fermentation; SSF, solid-state fermentation; TAG, triacylglycerol.

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Table 1
Sources of lipases from the genus *Penicillium*.

The genus *Penicillium*	References
P. abeanum	[9]
P. aurantiogriseum (formerly *P. cyclopium*)	[10–15]
P. avellaneum	[15]
P. brevicompactum	[15]
P. camembertii	[16–19]
P. caseicolum (*P. candidum*)	[15,20–23]
P. charlesii	[15]
P. chrysogenum	[24–26]
P. citrinum	[15,27–33]
P. corylophilum	[15]
P. corymbiferum	[15]
P. crustosum	[15]
P. duclauxii	[15]
P. egyptiacum	[15]
P. expansum	[15,34]
P. funiculosum	[15,35]
P. granulatum	[15]
P. griseofulvum	[15]
P. melinii	[36]
P. nelicum	[15]
P. nigricans	[15]
P. oxalicum	[15]
P. restrictum	[37–42]
P. roqueforti	[43,44]
P. rugulosum	[15]
P. simplicissimum	[45–50]
P. spiculisporum	[15]
P. variable	[15]
P. verrucosum	[51,52]
P. wortmanii	[53]
Penicillium sp.	[15,54,55]

1. Introduction

The use of enzymes created opportunities for developing a green, sustainable and modern industrial chemistry due to excellent specificity, being atom economic, mild reaction conditions, energy-saving process and simplicity. The global enzyme market increased stably from $1.0 billion in 1995 to $1.5 billion in 2000, and then to $2.2 billion in 2006 [1,2]. It was estimated by BCC research that the global sale would reach $2.7 billion by the end of 2012 [2]. And at least 75% of the industrial enzyme market was occupied by hydrolytic enzymes such as protease, lipase and amylase [3]. Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) not only possess the natural function of hydrolyzing triacylglycerol (TAG) to glycerol and free fatty acids, but also catalyze esterification, transesterification and ammonolysis in nonaqueous media. They are one of the most promising hydrolases with industrial potential. And as a matter of fact, wide usage of lipases was found in food, detergent, chemical and pharmaceutical industries [3,4].

Lipases are widely distributed throughout plants and animals, as well as in microorganism. However, the most commercially useful lipases are of microbial origin, mainly bacteria [5], yeast [6,7] and fungi [8]. The *Penicillia* are one of the common fungal producers of lipases, which are presented in Table 1. In addition, several lipases such as lipase G "Amano" 50® from *P. camembertii* and lipase R "Amano"® from *P. roqueforti* (produced by Amano Enzyme Inc., Japan), and lipase LVK® from *P. expansum* (produced by Shenzhen Leveking Bioengineering Co., Ltd., China) are commercially available.

In nature, the *Penicillia*, versatile and opportunistic fungi, are mostly saprophytic. They are post-harvest pathogens, which are one of the most common causes of fungal spoilage in fruits and vegetables. On the other hand, many species serve human. For example, *P. roqueforti* and *P. camembertii* have been used to make cheese for a long history [56]. In particularly, the *Penicillium* is famous as the producer of the first antibiotic penicillin. The original producer *P. notatum*, which was discovered by Fleming in 1928, was later replaced with *P. chrysogenum*, a more productive species [57].

The *Penicillia* are good producers of extracellular enzymes such as lipase, proteases, cellulases and xylanases, which was thoroughly reviewed by Hamlyn et al. in 1987 [58]. In 2006, Eyzaguirre and co-workers comprehensively summarized the xylanolytic enzyme system from the genus *Penicillium* [59]. Herein, we provided an update on the substantial progress of *Penicillium* lipases from 1988 to early-2010, especially focusing on their production, purification, characterization and applications.

2. Production

2.1. Submerged fermentation

Since aerobic submerged fermentation (SmF) was launched as a way of increasing the production capacity of penicillin during World War II in the 20th century, SmF has become a model technology for manufacturing fermented products. For example, approximately 90% of all industrial enzymes are produced by SmF [60]. Also, it was a dominant process for the production of the lipases from *Penicillia*.

Optimization of the fermentation conditions is an efficient route for improving lipase production, especially on carbon and nitrogen sources, C/N ratio, inducers, temperature, etc. For instance, Lima et al. isolated a wild fungal *P. aurantiogriseum* (formerly *P. cyclopium*) and investigated the effects of nitrogen and carbon sources on lipase production [14]. It was worth noting that the strain enabled to efficiently secrete the lipase with inexpensive $(NH_4)_2SO_4$ as the sole nitrogen source, since complex nitrogen sources such as yeast extract, peptones, soybean meal and corn steep liquor were required traditionally [10,27,29,37]. Bancerz et al. also found that the psychrotrophic fungus *P. chrysogenum* 9′ from the arctic tundra soil not only utilized various organic nitrogen sources among which urea was the best, but also took inorganic $NaNO_3$, giving just slightly lower lipase activity [24]. And Pimentel et al. reported yeast extract could be replaced by inexpensive 0.75% $(NH_4)_2SO_4$ complemented with mineral salts for lipase production [30]. Furthermore, the specific activity of the lipase was more than 1.5-fold higher than that in the medium with yeast extract. The morphology of the fungus changed totally in the media with the two different nitrogen sources.

In SmF, plant oil such as olive oil [14,21,28,29,37,53,54], Jojoba oil [19], corn oil [24] and cottonseed oil [36] generally worked as carbon sources as well as effective inducers for lipase production from *Penicillia*. And it was found that among numerous components in olive oil, the major oleate proved to be the responsible enhancer for the lipase biosynthesis [21]. But no lipolytic activity was detected when glucose acted as the sole carbon source [14,37], which was attributed to catabolic repression. Dai et al. found that 0.5% starch could be used as carbon source for the production of alkaline lipase from *P. expansum* PED-03 [34]. And Tween 80 was an effective inducer for producing lipase, which was also found in the production of lipase from *P. citrinum* [28].

Chahinian et al reported the production of lipase from *P. cyclopium* in shaken culture in the absence or presence of olive oil [10]. This strain could produce two types of lipases, lipase I and II showing preferential specificity toward TAGs and partial acylglycerols, respectively. Intriguingly, in the absence of olive oil, lipase I (25 U/mL) was favorably produced while the concentration of lipase II did not exceed 7 U/mL. On the contrary, in the presence of olive oil, the culture medium was almost devoid of lipase I and the concentration of lipase II reached 35 U/mL.

Also, Vanot et al. found that *P. cyclopium* isolated from moldy walnuts secreted preferentially lipase I in stationary batch culture whilst lipase II was produced favorably in shaken culture [11,12].

P. camembertii U-150 was capable of producing a unique lipase (MDGL), which hydrolyzed monoacylglycerols (MAGs) and diacylglycerols (DAGs) but not TAGs. Yamaguchi et al. reported the expression of MDGL in *Saccharomyces cerevisiae* and identification of the catalytic amino acid residues through site-directed mutagenesis [16]. Later, the same research group developed a new and efficient expression system (*Aspergillus oryzae*) for MDGL production, because of extensive glycosylation and low productivity of the lipase in *S. cerevisiae* [18]. Although it has been demonstrated that adding lipids is a general approach for inducing the secretion of lipases from some fungi [37,53], the authors attempted to elucidate the lipase expression through the gene regulation. It was shown that the region between the positions at −382 and −554 bp from the translation initiation point was important to the higher expression of MDGL gene, while the presence of introns presented no effect.

A strain *P. cyclopium* PG37 isolated by Sun and co-workers was able to produce an alkaline lipase with high lipolytic activity [61]. However, this lipase has a low homology with lipases from *P. camembertii* (22.9%), *Rhizomucor miehei* (22.3%) and *Thermomyces lanuginosus* (25.6%) in the primary structure. And its gene was successfully cloned with four steps of polymerase chain reaction (PCR) amplification and expressed in *Escherichia coli* by Huang and co-workers [13].

Wolski et al. observed that immobilization was a convenient and efficient approach for enhancing lipase production [55]. For example, the lipase activity (21 U/mL) was much higher by using immobilized cells than that (9.5 U/mL) by using free cells [54].

Industrial waste such as vegetable oil refinery residue [31] and olive mill wastewater (OMW) [32,33] was evaluated to be possible media for the production of the lipase from *P. citrinum*. Interestingly, the activity in the medium with vegetable oil refinery residue as carbon source was more than 2-fold higher than that with olive oil [31]. Moreover, the optimal nitrogen source was 0.74% NH_4Cl. It was estimated that 3×10^7 m^3 of OMW was generated annually in the three-phase olive oil extraction process in Mediterranean area, which was a serious environmental problem [62]. However, it was reported that OMW containing simple and complex sugars was a promising medium for lipase production [32]. *P. citrinum* NRRL 1841 was capable of producing lipase on four types of undiluted OMW with the total phenol concentration as high as 2.24–3.45 g/L [33]. It was a noteworthy result, since it was well known that OMW exerted concentration-dependent inhibitory effects on fungi, manly due to phenols [63]. The authors found that lipase production from *P. citrinum* NRRL 1841 in OMW-based media was remarkably enhanced by adding nitrogen source, in which NH_4Cl was the best. Response surface methodology was used to optimize three key parameters, namely initial pH and concentrations of NH_4Cl and yeast extract [33]. Maximum lipase activity (1230 U/L) was achieved after 188 h of incubation in shaken flasks, in which one unit was defined as the amount of enzyme producing 1 μmol product per minute at 35 °C using β-naftimyristate as the substrate. And stirred tank and bubble column reactor with working capacity of 3 L was examined for lipase production, resulting in lower lipase activities (735 and 430 U/L, respectively).

Forty *Penicillia* and 40 *Aspergilli* were screened for extracellular lipase production on tributyrin and Nile blue sulfate agar plates by Yadav et al. [15]. It was revealed that 29 *Aspergilli* and 26 *Penicillia* were lipase producer. Among them, 10 *Aspergilli* and 9 *Penicillia* were considered as the best; plus, 8 *Aspergilli* and 15 *Penicillia* were new. The authors proposed that a screening strategy for lipase-producing species should involve at least two plate assays. The standard of a good lipase producer was that it could form a sizeable zone on all the plates. Furthermore, titrimetry should be used for quantitative estimation.

2.2. Solid-state fermentation

Solid-state fermentation (SSF) is defined as the fermentation on moist solid substrate in the absence or near absence of free water, thus being close to the natural environment to which microorganisms are adapted [64]. In the last two decades, SSF has attracted increasing attention for the production of enzymes, metabolites, etc., due to several biotechnological advantages such as higher fermentation productivity, higher end-product concentration, higher product stability, and lower catabolic repression [60]. In 2000, Castilho et al. presented an economic evaluation of lipase production from *P. restrictum* in SSF and SmF [65]. For a 100 m^3/year scale, total capital investment in SmF was 78% higher than that in SSF. Additionally, SSF seemed to be more attractive from the economical viewpoint, because the unitary lipase cost was 47% lower than the selling price in SSF, while 68% higher than that in SmF. More importantly, SSF offers a useful tool for processing agro-industrial residues.

Gombert et al. reported that babassu cake, solid waste from babassu oil industry, was used as the basal medium for lipase production from *P. restrictum* in SSF [40]. The supplementation of nutrients such as peptone, olive oil or starch was necessary for efficient fermentation. Also, the type and the amount of supplemented nutrients were crucial for lipase production. Small changes in C/N ratio with the addition of carbon or nitrogen sources led to great variations in enzyme activities. The highest lipase activity (30.3 U/g of initial dry weight) was obtained after 24 h of fermentation with addition of 2% olive oil.

Freire and co-workers compared the effect of carbon sources on lipase production from *P. restrictum* in SmF and SSF [42]. Higher lipase activities were obtained after a shorter fermentation time (24 h) in SSF with babassu cake as basal solid medium, as compared to those in SmF. Interestingly, the negative regulatory effect, caused by glucose in lipase production in SmF, was not observed in SSF. The capacity of SSF system to significantly overcome catabolic repression was ascribed to various physicochemical factors and specific culture conditions. Previous reports also described the ability of SSF to reduce catabolic repression in the production of other hydrolases [66–68].

Similarly, with babassu cake as basal solid medium, Gutarra et al. studied the lipase production from *P. simplicissimum* in tray-type and packed bed bioreactor [46]. After the optimization of the culture conditions through response surface methodology, maximum lipase activity obtained in packed bed bioreactor were 30% higher than that in tray-type reactor. Then, the same research group developed a powerful inoculation strategy for the lipase production from *P. simplicissimum* in SSF [47]. Traditional spore inoculation was compared to inoculation with fungal pellets grown in liquid medium and fermented cake (a small-scale SSF inoculated with spores). Fermented cake inoculation appeared to the best, yielding a productivity of 0.45 U/(g h), which was comparable to the highest values obtained with conventional spore inoculation. Upon optimization, the lipase activity and productivity reached 30 U/g and 0.63 U/(g h), respectively. The new inoculation strategy required 10 times less spores and furnished 1.5-fold higher lipase activity and productivity as compared to conventional spore inoculation, demonstrating its application potential for SSF.

Soybean meal, an agro-industrial byproduct, was also tested as the basal solid medium for *P. simplicissimum* lipase production [45,48]. In addition, it was reported that *P. verrucosum* was capable

Table 2
Purification protocols for the lipases from the genus *Penicillium*.

Source	Purification technique	Purification factor[a]/yield[b]	Reference
P. abeanum	Ultrafiltration, DEAE Sephadex A-50, Sephadex G-100, Ether Toyopearl 650M	200-fold/12%	[9]
P. camembertii U-150	Ethanol precipitation, $(NH_4)_2SO_4$ precipitation, DEAE Sephadex CL-6B, Cellulofine GC-700m, Mono-Q HR 5/5 (FPLC), Mono P HR 5/20 (FPLC)	210-fold/2.6%	[73]
P. camembertii U-150	Ethanol precipitation, $(NH_4)_2SO_4$ precipitation, DEAE-Sepharose, Aminooctyl-Sepharose, Hydroxyapatite column, ConA-Sepharose	213-fold/27%	[74]
P. camembertii Thom PG-3	pH precipitation, ethanol precipitation, $(NH_4)_2SO_4$ precipitation, DEAE-Cellulose	22-fold/8.7%	[19]
P. candidum	$(NH_4)_2SO_4$ precipitation, Octyl Sepharose CL-4B, DEAE Sephadex A-50	37-fold/0.8%	[22]
P. chrysogenum	Ultrafiltration, Phenyl-Sepharose CL-4B, Mono-Q HR 5/5, PD-10 (Sephadex G-25 M)	30.3/44%	[75]
P. cyclopium	$(NH_4)_2SO_4$ precipitation, Sephadex G-25, DEAE-Sephacel A-50, Sephadex G-100,	94-fold/7.0% 22-fold/3.0%	[76]
P. cyclopium lipase I	$(NH_4)_2SO_4$ precipitation, Sephadex G-75, DEAE-Sephadex, Sephadex G-75	93-fold/35%	[77]
P. cyclopium lipase II	$(NH_4)_2SO_4$ precipitation, Sephadex G-75, DEAE-Sephadex, Sephadex G-75	590-fold/30%	[10]
P. cyclopium PG37	$(NH_4)_2SO_4$ precipitation, phenyl-Sepharose CL-4B, DEAE-Sepharose, Sephadex G-75	16.5-fold/33.2%	[61]
P. citrinum	$(NH_4)_2SO_4$ precipitation, Superose-6 HR 10/30, Phenyl Superose HR 5/5	379-fold/15.2%	[78]
P. citrinum	$(NH_4)_2SO_4$ precipitation, AOT/isooctane reversed micelles, Phenyl Superose	810-fold/68%	[79]
P. expansum PED-03	DEAE-Sepharose, Sephacryl S-200	81.8-fold/19.8%	[80]
P. roqueforti IAM 7268	Ethanol precipitation, $(NH_4)_2SO_4$ precipitation, DEAE-Toyopearl 650M, Phenyl-Toyopearl 650M, Toyopearl HW-60	17-fold/32%	[43]
Penicillium sp. UZLM-4	Acetone precipitation, Q-Sepharose, butyl-Sepharose, Mono-Q (FPLC)	18-fold/21.9%	[81]

[a] The ratio of specific activity of the purified lipase to the initial specific activity.
[b] The percentage of total activity of the purified lipase in the initial total activity.

of using soybean bran as the substrate for the lipase production [51]. Fermentation conditions were optimized through response surface methodology. It was shown that temperature and moisture were the key factors.

Godoy described a simultaneous and efficient approach for detoxification of castor bean waste and production of an acidic and thermostable lipase [49]. Castor bean waste, the byproduct of castor oil extraction, presents no utility, because of being extremely alkaline, toxic and allergenic [69,70]. Hence, it would cause a significant environmental problem. It was noteworthy that *P. simplicissimum* enabled to grow and produce lipase on castor bean waste. After optimization, the maximum lipase activity of 44.8 U/g (one unit: the enzyme amount that releases 1 μmol product per minute at pH 7.0 and 30 °C using *p*-nitrophenyl laurate as the substrate) was achieved, which was much higher than those with the same strain, but different wastes [45,46]. Moreover, the strain was capable of degrading ricin completely and decreasing the allergenic potential by about 16%.

3. Purification

Although most of commercial enzyme preparations need not be purified to homogeneity, a certain extent of purity allows successful usage in the industries. In addition, the purification of the enzymes is indispensable for assaying their primary amino acid sequence or three-dimensional structure, and understanding the structure–function relationships [5,71,72].

As shown in Table 2, the lipases from the genus *Penicillium* were purified by various protocols, furnishing diverse purification factors and yields. Several interesting examples were presented in the rest of this section.

The extraction of the lipase from the solid medium in SSF was reported by two groups [52,82]. The lipase recovery was improved remarkably upon optimization using response surface methodology. Recently, Cunha et al. reported that the separation was coupled with the immobilization of the lipase from *P. simplicissimum* by selective adsorption on hydrophobic supports [83].

Krieger et al. described a three-step strategy for lipase purification from *P. citrinum* [78]. At the beginning, a three-step protocol was employed, consisting of $(NH_4)_2SO_4$ precipitation, ion exchange chromatography on a Mono-Q HR 5/5 column and gel filtration chromatography on a Superose 6 HR 10/30 column. It gave aggregated lipase complex with lower activity and a lower yield of 24.4%. Thus, an alternate strategy was used, in which $(NH_4)_2SO_4$ precipitation was followed by gel filtration chromatography on a Superose 6 HR 10/30 column (69.3% yield) and hydrophobic interaction chromatography on a Phenyl Superose column (15.2% yield). The dimer of the lipase was achieved. The gel filtration step was scaled up by using a similar column (a Superose 6 XK 26/70 preparative column) on a BIO-PILOL system owing to low protein loading capacity of the Superose 6 column of the FPLC system. It gave a yield of 54.8%.

Although the gel filtration step could be scaled up, hydrophobic interaction chromatography was not always effective in the disruption of the aggregated complex; so, it was difficult to obtain the monomer with higher activity [78]. The same group proposed the usage of sodium di(2-ethylhexyl)sulphosuccinate (Aerosol OT, AOT) reversed micelles for purifying *P. citrinum* lipase, which gave a better activity yield (68%) and the monomer protein [79]. It comprised the extraction and back-extraction of the lipase from the crude extract by using AOT revered micelles in isooctane. Surprisingly, no enzyme activity could be detected either in the micellar phase or in the aqueous phase after back-extract. However, the lipolytic activity was recovered after hydrophobic interaction chromatography on a Phenyl Superose column.

Table 3
General biochemical properties of lipase from the genus *Penicillium*.

Source	Molecular mass (kDa)	p*I*	pH/temperature (°C) optimum	Stability	Reference
P. abeanum	28	3.4	7–8/25–30	Maintain initial activity after 16 h at pH 7.0–8.5, 25 °C	[9]
P. camembertii U-150	39	–	–/–	–	[73]
P. camembertii Thom PG-3	28.18	–	8.0/48	–	[19]
P. candidum	29	5.5	9.0/35	100% and 70% residual activity after 10 min at <25 and 35 °C, respectively	[22]
P. caseicolum	–	–	9.0/35	Maintain initial activity for 1 month at −15 °C; 98% residual activity for 72 h at 25 °C	[23]
P. chrysogenum 9′	–	–	5.0/30	100% and 50% residual activity for 60 min at 20 and 40 °C, respectively	[24]
P. citrinum	–	–	8.0/34–37	Stable in a pH range 6.0–7.0; stable for at least 2 h at <45 °C	[29]
P. citrinum	33	4.8–5.0	8.0/45	–	[78,79,88]
P. chrysogenum	40	3.8	7.9–8.1/45	Stable at 25 °C	[75]
P. aurantiogriseum	–	–	8.0/60	32%, 45% and 77% residual activity after 30 min at 50, 45 and 37 °C, respectively	[89]
P. cyclopium	93	5.2	7.0/37	50% residual activity after 4 years at 4 °C	[76]
P. cyclopium lipase I	29	–	8–10/–	90% residual activity after 1 h at pH 8 and 35 °C; lost activity after 10 min at 45 °C	[77]
P. cyclopium lipase II	40–43	–	7.0/40	Stable in a temperature range of 25–40 °C at pH 7.0; stable in a pH range of 4.5–7.0;	[10]
P. cyclopium PG37	29	–	10.0/25	100% and 30% residual activity after 20 min at 30 and 40 °C, respectively	[61]
P. cyclopium PG37 (recombinant)	33	–	10.0/30	–	[13]
P. expansum PED-03	28	–	9.5/35	Stable within the range of pH 7.0–10.0	[34,80]
P. expansum DSM 1994	25	5.5	9.0/45	Stable within the range of pH 6.0–10.0	[90]
P. expansum PF898	28	–	9.5/34	–	[87]
P. roqueforti IAM 7268	25	7.2	6.0–7.0/35	Stable for 15 min from pH 6.0–8.0 at 30 °C	[43]
P. simplicissimum	–	–	4.0–5.0/50	A half-life of 5.0 h at pH 5.0 and 50 °C	[50]
P. sp. UZLM-4	27	–	–	–	[81]
P. sp.	–	–	7.0/37	Maintain initial activity for 60 days at −10 °C	[55]
Penicillium sp.	–	–	4.9–5.5/37–42	Maintain initial activity up to 100 days at freezing temperature	[54]
P. verrucosum	–	–	7.0/44	Stable at pH 6.0–7.0, 35 °C; maintain initial activity for 56 days at freezing temperature	[51]
P. verrucosum	–	–	8.5/42	Maintain initial activity after 91 days at both 4 and −10 °C	[52]
P. wortmanii			7.0/45	55% residual activity after 1 h at 50 °C	[53]

4. Characterization

4.1. General biochemical properties

Lipases from *Penicillium* have been characterized extensively. The general biochemical properties of the lipases including molecular mass, p*I*, optimum pH, optimum temperature and stability were summarized in Table 3. The specificity such as substrate and position specificity, effects of metal ions, surfactants and inhibitors, activity and stability in organic solvents were presented in the following subsections.

Isobe et al. [84,85] reported the complete amino acid sequence of MDGL from *P. camembertii* U-150, which was known as unique substrate specificity toward partial acylglycerols [73]. This lipase has a single polypeptide chain consisting of 276 amino acid residues with two short disulfide bridges (Cys36-Cys41 and Cys103-Cys106). The catalytic triad of Asp, His and Ser, was conserved at positions 199, 259 and 145, respectively.

Although MDGL was similar pronouncedly in the gene sequence to *T. lanuginosus*, its thermostability was much lower than that of *T. lanuginosus* lipase [74,85,86]. Yamaguchi et al. proposed that the reason was the lack of characteristic long disulfide bridge in this lipase, corresponding to Cys22-Cys268 in *T. lanuginosus* lipase. Hence, the authors established a disulfide in MDGL via double mutant (Y22C and G269C) [86]. As expected, the melting temperature of the lipase was increased from 51 to 60 °C by the extra disulfide bond. Unfortunately, the engineered disulfide link was labile, since no difference was observed under reducing conditions.

Wu et al. [61] determined the *N*-terminal amino acid sequence (NH_2-Ala-Thr-Ala-Asp-Ala-Ala-Phe-Pro-Asp) of the lipase from *P. cyclopium* PG37, and found that it had no sequence homology compared with other lipases such as MDGL abovementioned [85].

Bian et al. [87] reported the gene sequence of lipase from *Penicillium expansum* PF898 (Genebank access number AA330635). The mature peptide of the lipase has 258 amino acid residues. And the amino acid residues 130–136 (GHSLGGA) are highly conserved compared with other lipases, in which catalytic serine presents. The lipase contains 4 cysteine residues (Cys25, Cys220, Cys228 and Cys254), and the author proposed that 2 possible disulfide bonds might present. No *N*-glycosylation site was found in the lipase. A unique feature of this lipase is its high percentage of hydrophobic residues (51.4%) in the *N*-terminal region compared with other typical lipases.

4.2. Specificity

Specificity is one of the most important properties of enzymes, mainly including substrate and position specificity. The cell-bound lipase from *P. cyclopium* was specific toward short-chain TAG, especially tributyrin (C4) [76], as observed in the cases of lipases from *P. chrysogenum* 9′ [24], *P. chrysogenum* [75], *Penicillium* sp. UZLM-4 [81], and *P. citrinum* [91]. Although lipase from *P. candidum* also displayed the highest activity toward tributyrin among various TAGs tested [22,23], long-chain palmitate (C16) was the best for the lipase when *p*-nitrophenyl fatty acid esters were used as the substrates [22]. On the contrary, lipase from *P. roqueforti* IAM 7268

showed high specificity toward short-chain fatty acid esters such as butyrate (C4) and caproate (C6) with *p*-nitrophenyl esters as the substrates [43].

Sugihara et al. reported that *P. abeanum* lipase showed lower activities toward ester bonds of polyunsaturated fatty acid esters as compared to those of other esters [9]. Moreover, it displayed preferentially activities toward medium-chain fatty acid esters, which was also found in the case of lipases from *P. camembertii Thom* PG-3 [19], *P. aurantiogriseum* [89], *P. cyclopium* [10] and *P. simplicissimum* [50]. However, MDGL from *P. camembertii* U-150 hydrolyzed long-chain MAGs most efficiently [73]. And more interestingly, it was strictly specific toward MAGs and DAGs, and was inactive completely toward TAGs. Likewise, lipase II from *P. cyclopium* hydrolyzed MAGs and DAGs but not TAGs while lipase I from the same strain predominantly favored TAGs [10,92].

Most of lipases from *Penicillia* have a hydrolytic preference for 1- and 3-positions of TAGs, furnishing 1,2-DAGs [9,19,76,81]. For example, *P. abeanum* lipase hydrolyzed the ester bond of 1- or 3-position 9-fold faster than 2-position of triolein [9]. Although MDGL from *P. camembertii* U-150 was capable of hydrolyzing 2-MAGs, its hydrolytic conversions were lower as compared with those of the corresponding 1(3)-MAGs [73]. But lipases from *P. chrysogenum* 9′ and *P. expansum* PF898 were not able to discriminate between 1- and 2-positions of TAGs [24,87]. Interestingly, it was found that *P. citrinum* lipase preferred the 2-position of triolein to 1-position [91].

4.3. Effects of metal ions, surfactants and inhibitors

Stimulatory or inhibitory effects of metal ions on the lipase activity have been well established [5], although cofactors, in general, are not required for the catalytic activity. The influence of metal ions varied with the sources of lipases. For example, *P. chrysogenum* 9′ lipase was slightly activated by Ca^{2+} and Mn^{2+}, while Sn^{2+}, Zn^{2+} and Cu^{2+} caused moderate inactivation (about 20–30%) [24]. Lipase from *P. abeanum* was inhibited strongly by Hg^{2+}, suggesting the presence of key cysteine residue(s) [9]. And other ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Co^{2+}, Mo^{2+}, Ba^{2+} and Zn^{2+} decreased the activity by 10% or less. In the case of *P. candidum* lipase, Ca^{2+} at 1 mM was the only metal ion with a positive effect on the activity. The activity of *P. aurantiogriseum* lipase was marginally enhanced by Mg^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}, whereas Cu^{2+}, Ba^{2+} and Ca^{2+} showed negative effects on the activity [89], in spite of the general stimulatory influence caused by Ca^{2+} [22,24]. Like *P. abeanum* lipase [9], Hg^{2+} was a strong inhibitor for the lipase [89]. It was reported that *P. camembertii* MDGL was inhibited significantly by Hg^{2+} as well as Fe^{3+} [10]. Mase et al. reported that lipase from *P. roqueforti* IAM 7268 displayed high tolerance toward NaCl [43].

Surfactants are generally used for the preparation of emulsion in assaying lipase activity and also in the purification. They could destroy the tertiary structures of enzymes, leading to denaturation. On the other hand, the aggregation of enzymes would be relieved considerably by using these compounds, thus enhancing enzyme activity. The influence of various surfactants on the lipase activity varied greatly [22,93]. For example, Ibrik et al. described the influence of surfactants such as sodium taurodeoxycholate, sodium taurocholate and Triton X-100 on the activity of *P. cyclopium* lipase with tributyrin and olive oil as model substrates [77]. The activity was not affected by sodium taurodeoxycholate at concentration less than 8 mM in the hydrolysis of both model substrates, whereas it was totally inhibited by Triton X-100, Brij 35, Tween 20 and 80. Furthermore, the effect of sodium taurocholate on the activity depended on the substrates. Similarly, Triton X-100, sodium taurocholate, Cetrimide, Tween 20 and CHAPS were strong inhibitors for lipase from *P. chrysogenum* 9′, except for cholic acid enhancing lipase activity [24]. In addition, the lipase was drastically inhibited by adding phenylmethylsulphonyl fluoride and EDTA, but eserine (esterase inhibitor), *o*-phenantroline (zinc-specific chelator), iodoacetamide and *N*-ethymaleimide had no effect on the enzyme activity. Lipase from *P. aurantiogriseum* was found to be stable in 0.01% Tween 80 and 0.01% sodium taurocholate after 1 h at 28 °C [89]. Nevertheless, the lipase lost approximately 59% of its initial activity after the treatment of 0.01% Triton X-100. No significant change of the activity of MDGL was observed upon addition of varied compounds such as EDTA, iodoacetic acid and phenylmethylsulphonyl fluoride [73].

4.4. Activity and stability in organic solvents

High activity and stability of the lipase in organic solvents is desirable for its application in organic synthesis. Lima et al. reported the stability of crude lipase from *P. aurantiogriseum* against several organic solvents [89]. The lipase was very tolerant toward hydrophobic solvents such as hexane, *n*-heptane, and isooctane. After 1 h at 28 °C, the residual activities were 113.6% in *n*-heptane, 92.3% in hexane, and 91.0% in isooctane, respectively. However, no or near no activity was found in polar solvents such as methanol, ethanol, *i*-propanol, acetone and *n*-butanol. Interestingly, the activity of *P. chrysogenum* 9′ lipase was greatly enhanced by adding DMSO at concentrations below 30% [24]. The lipase almost maintained its initial activities after 24 h of incubation in the presence of hexane and dioxane at a 50% concentration, whereas it was amenable to denaturation in xylene-containing system. Recently, Yang et al. reported that hydrolytic activity of lipase from *P. expansum* was enhanced by 12 times when organic solvent was replaced by the ionic liquid [BMIm]PF_6 [94].

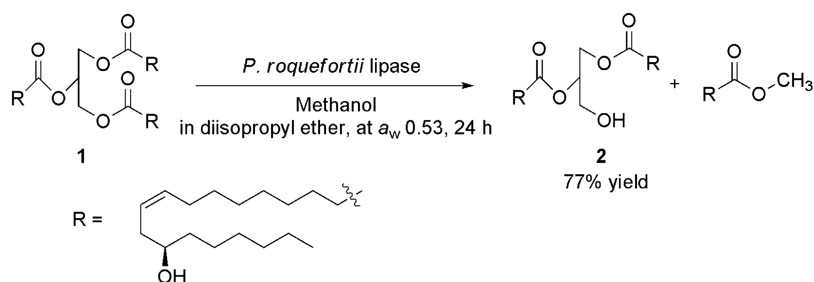
5. Applications

5.1. In the modification of oil and fat

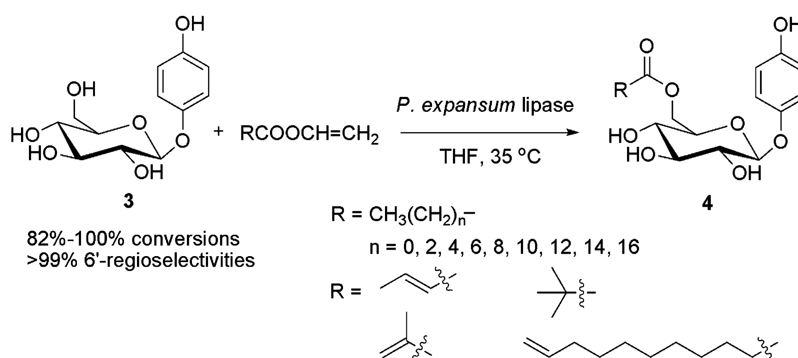
MAGs and DAGs are of importance as emulsifiers in foods, cosmetics and pharmaceuticals. In addition, they are useful building blocks for the synthesis of phospholipids, glycolipids and some prodrugs such as 1,2-DAG conjugated (*S*)-(3,4-dihydroxyphenyl)alanine (L-Dopa) for treatment of Parkinson's disease [95]. Fureby et al. described the preparation of 1,2-dilaurin via alcoholysis of trilaurin catalyzed by *P. roqueforti* lipase [96]. Low yields and low regioselectivities were obtained with aliphatic hydrocarbons as solvents, presumably due to acyl migration [97]. Ethers such as diisopropyl ether and methyl *t*-butyl ether were the best solvents. The lipase displayed much higher activities for trilaurin and monolaurin than that for dilaurin and a clear preference for 1(3)-position. Upon optimization, a yield of 75% was achieved, and the content of 1,2-dilaurin was 95% in the product mixture.

Likewise, Turner et al. reported *P. roqueforti* lipase-catalyzed methanolysis of triricinolein **1** to synthesize 1,2(2,3)-diricinolein **2** in diisopropyl ether (Scheme 1) [98]. The influence of water activity (a_w) on the enzymatic reaction was subtle. For example, a higher yield of diricinolein (93%) was afforded at a_w of 0.11 as compared to that (88%) at a_w of 0.53. Whereas, the purity of 1,2(2,3)-diricinolein **2** were 71% and 88% at a_w of 0.11 and 0.53, respectively.

Yamaguchi et al. reported the synthesis of MAGs as the major product and DAGs via *P. camembertii* lipase-mediated esterification between fatty acids and glycerol with high yields [99]. It was found that long-chain (C18) unsaturated and medium-chain (C10 and 12) saturated fatty acids were good acyl donors. Under optimal conditions, the conversion was up to 76% and the formed glyceride contained more than 90 wt% MAG. In addition, the conversion was increased by adding molecular sieves. When fatty acid vinyl



Scheme 1. Methanolysis of triricinolein catalyzed by lipase from *P. roqueforti*.



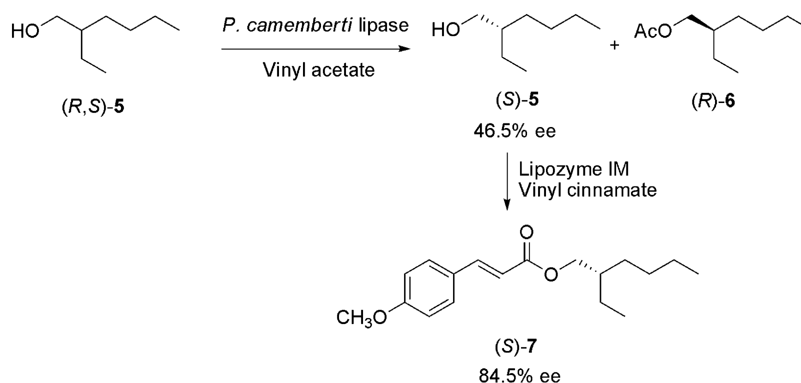
Scheme 2. Synthesis of arbutin esters through enzymatic regioselective acylation.

ester was used as acylating agent, MAG was obtained with a higher yield.

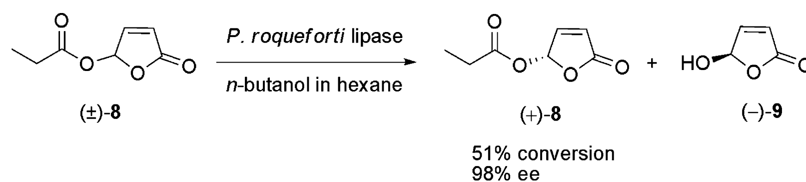
Polyunsaturated fatty acids are of physiological importance for humans, such as relieving atherosclerosis and enhancing immune function [100]. Torres described the esterification of glycerol with conjugated linoleic acid (CLA) and long-chain fatty acids from menhaden oil catalyzed by the lipases [101]. As compared with those from *Pseudomonas cepacia*, *Candida antarctica*, and *Mucor miehei*, lipase from *P. camembertii* gave a much slower reaction rate and a poor yield. Nevertheless, Otero and co-workers observed that lipase from *P. camembertii* was a good catalyst for the synthesis of DAG from CLA and partial glyceride comprising 10 wt% DAG and 90 wt% MAG [102]. After 4.5 h, 98.6 wt% DAG and 1.4 wt% MAG were obtained, and more than 95% unsaturated fatty acid residues were present in DAG.

Watanabe et al. describe a two-step successive enzymatic reaction for the synthesis of MAG of CLA [103]. The first step was *P. camembertii* lipase-catalyzed esterification between CLA and glycerol for 10 h at 30 °C, affording a CAL conversion of 84%. The reaction was continually conducted at 5 mmHg for dehydration, furnishing an increasing conversion of 95% after another 24 h. The reaction mixture contained 50 wt% MAG and 44 wt% DAG. The second step was enzymatic glycerolysis of the obtained DAG. The mixture from the first step was solidified on ice and incubated at 5 °C. The content of MAG increased to 88.6 wt% in the mixture after 15 days. In order to reduce the long reaction time of this process (about 2 weeks), the same research group improved it by combining low temperature with low vacuum [104]. Enzymatic esterification with the molar ratio (1/5) of CLA to glycerol for 20 h at 5 °C (80.8% conversion) was followed by dehydration at 5 mmHg for another 16 h. The final conversion arrived at 94.5%. And in the reaction mixture, the contents of MAG and DAG were 92.7 wt% and 2.9 wt%, respectively.

Also, they proposed there was a critical temperature for the production of MAG [105], under which MAG was solidified and excluded from the solution, thus failing to be further biotransformed to DAG. They investigated the influence of temperature on the synthesis of MAG of C10–C18 free fatty acids, and determined the critical temperatures for each MAG. Indeed, it was found that the critical temperature for the synthesis of each MAG showed a linear correlation with its melt point. It provided a convenient approach to predict the optimal temperature for the synthesis of MAG.



Scheme 3. Enzymatic synthesis of (*S*)-2-ethylhexyl *p*-methoxycinnamate.



Scheme 4. Enzymatic kinetic resolution of the racemic mixture (±)-**8**.

Although the reaction time was shortened significantly [104], the improved process still had some drawbacks such as low temperature (large energy consumption) and high amount (5 equivalents) of glycerol. So Shimada and co-workers made a further improvement of the process for the production of MAG [106]. The reaction mixture was incubated at 30 °C and 5 mmHg with CLA/glycerol (1/2) in the presence of *P. camembertii* lipase. After 72 h, the conversion reached 95%, and the contents of MAG and DAG were 90 wt% and 6 wt%, respectively.

Sugihara et al. used *P. abeanum* lipase to enrich docosahexaenoic acid in Tuna oil [9]. The lipase showed lower activities toward ester bonds of polyunsaturated fatty acid esters compared with those of other fatty acid esters. After the enzymatic reaction, the content of docosahexaenoic acid in Tuna oil increased by more than 2-fold.

Our research group described the immobilization of *P. expansum* lipase and its application to produce biodiesel from corn oil in organic solvents [107]. A three-step methanolysis protocol was used, in which one molar equivalent of methanol was added at the beginning, 2 and 6 h, respectively. After 24 h of reaction, a methyl ester yield of 85% was obtained. The immobilized lipase retained 62.8% of its initial activity after reused for 10 batches. We also used waste oil with high acid value as the feedstock to produce biodiesel catalyzed by immobilized lipase from *P. expansum* [108]. The methyl ester yield could be enhanced remarkably by adding adsorbents, due to the removal of water produced by the esterification between free fatty acid and methanol. Silica gel was demonstrated to be the best among the adsorbents tested. After 7 h, a methyl esters yield of 92.8% was afforded through a similar three-step methanolysis approach. Recently, Yang et al. described the production of biodiesel from corn oil mediated by crude lipase from *P. expansum*-mediated transesterification in $[BMIm]PF_6$ [94]. The enzymatic transesterification was enhanced greatly using $[BMIm]PF_6$. A much higher methyl ester yield (69.7%) was furnished in the ionic liquid after 25 h, as compared to the yields of 19.4%, 14.0%, and 1.0% obtained in *t*-butanol, solvent-free system, and hexane, respectively.

5.2. In the modification of biologically active compounds

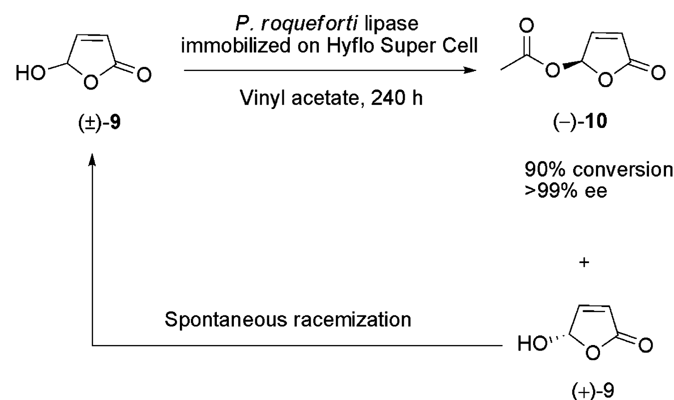
Kojic acid, 5-hydroxy-2-(hydroxymethyl)-1,4-pyrone, possesses inhibitory activities against mushroom and plant polyphenol oxidases, and tyrosinases [109]. Kojic acid works widely as a food additive for preventing enzymatic browning, and in cosmetic preparations as a skin-lightening or bleaching agent [110]. However, it is hydrophilic and unstable in cosmetic use. In order to improve its lipophilicity, Liu et al. reported the synthesis of kojic acid esters via lipase-catalyzed esterification [111]. Lipase from *P. camembertii* proved to be the best catalyst for the production of kojic acid monooleate. The effects of several variables such as organic solvents, acyl donors, and metal salts on the enzymatic reaction were explored. Under optimal conditions, the yield of kojic acid monooleate reached about 40%. After reaction at 40 °C for 10 days, the lipase retained 57% of its initial activity.

Arbutin **3**, *p*-hydroxyphenyl β-D-glucopyranoside, acted as a cosmetic whitening agent, and an antitussive, an agent for lowering blood sugar. However, this compound suffered from low bioavailability due to its poor membrane penetration. Our research group attempted to introduce a lipophilic group into the parent compound **3** to enhance its membrane penetration via enzymatic regioselective acylation (Scheme 2) [112]. A group of fatty acid ester derivatives **4** were successfully synthesized by immobilized lipase from *P. expansum* with good conversions (82%–100%) and high 6′-regioselectivities (>99%). In addition, the lipase was specific toward the medium-chain fatty acid vinyl esters, particularly vinyl octanoate.

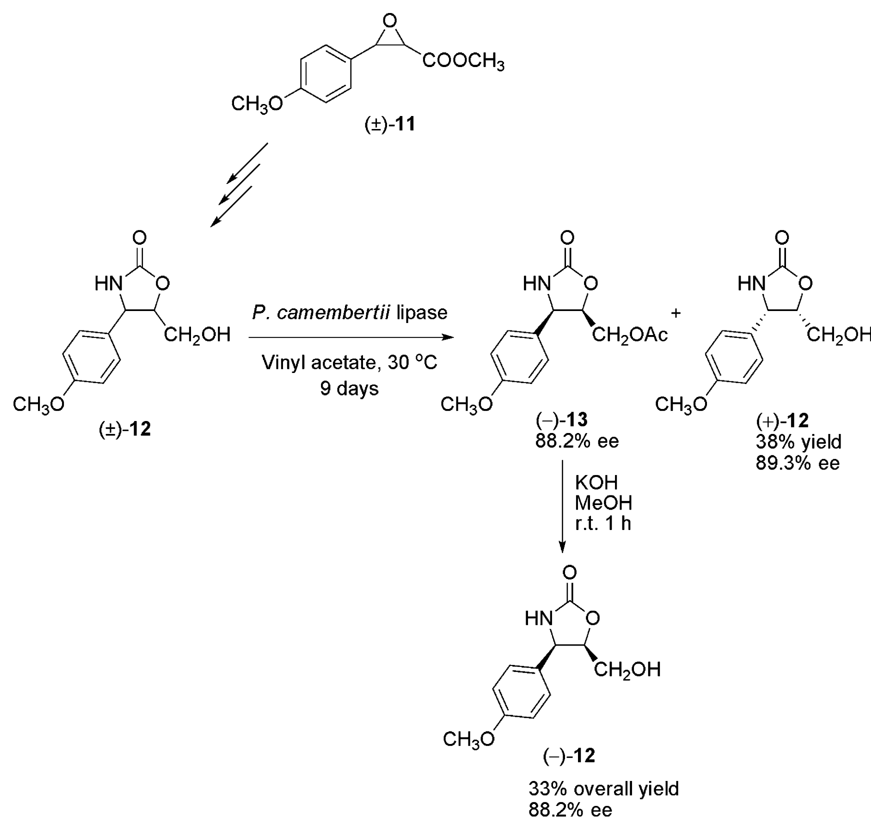
5.3. In the resolution of the racemic mixtures

Lipases have been demonstrated to be one of the most useful catalysts for the resolution of the racemic alcohols, amines and acids due to exquisite enantioselectivity [113]. For example, Majeric et al reported the preparation of (*S*)-2-ethylhexyl *p*-methoxycinnamate (*S*)-**7** via lipase-catalyzed sequential kinetic resolution (Scheme 3) [114]. In the first step, *P. camembertii* lipase was used for the resolution of racemic 2-ethylhexanol (*R*,*S*)-**5** with vinyl acetate, in which (*R*)-**5** was acylated preferentially, leaving (*S*)-enantiomer untouched. After 4.5 h, the conversion reached 79.8% with the (*S*)-**5** ee of 46.5% ($E = 3.0$). The product mixture without further purification was subjected to acylation with vinyl cinnamate in the presence of Lipozyme IM. The desirable product (*S*)-**7** was obtained with 84.5% ee.

Kellogg and co-workers reported the successful resolution of the racemic mixture (±)-**8** with *n*-butanol in the presence of *P. roqueforti* lipase (Scheme 4) [115]. After 24 h of incubation, conversion reached 51% and (+)-**8** was obtained with 98% ee ($E > 130$). The classical kinetic resolution has a limitation that the maximum theoretical yield is 50%. So the same group continued to improve the process by using dynamic kinetic resolution, thus allowing up to 100% conversion (Scheme 5) [116]. The ee of (−)-**10** was more than 99% at 90% conversion after 240 h by using immobilized *P. roqueforti* lipase. In addition, the reaction rate was increased dramatically in the presence of immobilized lipase from *Pseudomonas fluorescence* on Hyflo Super Cell, affording 100% conversion and slightly lower ee (89%) after 21 h.



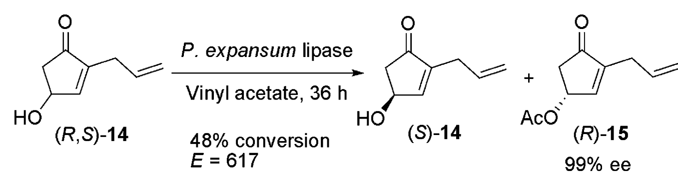
Scheme 5. Dynamic kinetic resolution of (±)-**9** catalyzed by immobilized lipase.



Scheme 6. Chemoenzymatic synthesis of enantiopure cytoxazone.

Hamersak et al. described a chemoenzymatic approach for preparing enantiopure cytoxazone **12**, in which the key step was enzymatic kinetic resolution (Scheme 6) [117]. A four-step chemical route started from easily available glycidic ester (±)-**11**, furnishing (±)-**12**. Then, enzymatic enantioselective acylation was performed with vinyl acetate in the presence of *P. camembertii* lipase at 30 °C. After 9 days of reaction, at 51% conversion, acetate (−)-**13** was achieved with 88.2% ee, and its enantiomer (+)-**12** was obtained with 38% yield and 89.3% ee. Acetate (−)-**13** was subjected to chemical hydrolysis, affording (−)-**12** with 33% overall yield.

An enzymatic resolution of racemic allethrolone (*R*,*S*)-**14** through transesterification reaction was described by Xia and co-workers (Scheme 7) [34]. Lipase from *P. expansum* PED-03 displayed high activity and excellent enantioselectivity ($E = 617$) in the enzymatic resolution. After 36 h, the conversion reached 48% and the product (*R*)-**15** ee was as high as 99%. Resolution of (*R*,*S*)-ibuprofen was also attempted with 1-propanol by using the same lipase in isooctane [80]. The lipase favored acylating (*S*)-ibuprofen, leaving (*R*)-enantiomer unreacted. After 48 h, the conversion reached 46% with an excellent enantioselectivity ($E = 536$). Furthermore, the same research group reported the immobilization of lipase from *P. expansum* PED-03, which was used for the resolution of (*R*,*S*)-**2**-octanol with vinyl acetate [118]. The enzyme activity and enantioselectivity (*E* value 560 vs. 11) of the lipase were enhanced remarkably by the immobilization on



Scheme 7. Enzymatic resolution of racemic allethrolone.

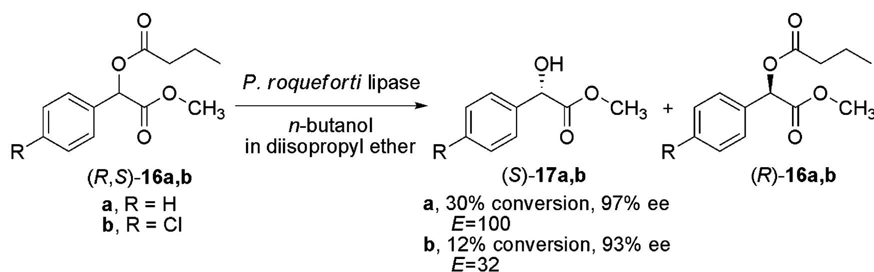
modified ultrastable-Y molecular sieves. After 24 h, the conversion reached 48% with excellent enantioselectivity ($E = 560$). Additionally, the immobilized enzyme displayed high operational stability. The average conversion was as high as 47% with excellent enantioselectivity (average $E > 460$) after reused for 8 batches (24 h/batch).

Miyazawa et al. reported enzymatic resolution of racemic *O*-butyryl mandelate (*R*,*S*)-**16** through *P. roqueforti* lipase-mediated alcoholysis [119]. The lipase displayed high enantioselectivity ($E > 30$). The desired products (*S*)-**17a** with 97% ee and (*S*)-**17b** with 93% ee were obtained with the conversions of 30% and 12%, respectively (Scheme 8).

5.4. In the pretreatment of wastewater with high oil and grease content

Wastewater from dairy and slaughterhouse contains oil and grease in significant amount, which is not prone to degradation. Direct disposal of this type of wastewater by conventional anaerobic processes would cause serious problems [120]. For example, lipids tend to adhere to the sludge surface, thus limiting the transport of soluble substrates to the microorganism and reducing operational efficiencies. In addition, it results in production of foam and unpleasant odors. It has been well established that the efficiencies of anaerobic bioreactor enable a remarkable improvement by complementing with the pretreatment catalyzed by the lipases [121–124]. In 2006, Cammarota and Freire presented a comprehensive review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content [120]. As a result, we just summarize the literature from 2006 to early-2010 in this subsection.

Leal et al. described anaerobic treatment of synthetic model dairy wastewater with and without pre-hydrolysis by solid enzyme preparation (SEP) from *P. restrictum* [125]. It was found that the performance of anaerobic treatment of wastewater with and without pre-hydrolysis was almost comparable when the con-



Scheme 8. Enzymatic resolution of racemic *O*-butyryl mandelate.

centration of oil and grease was blow 600 mg/L. However, when the concentration was up to 1000 mg/L, the wastewater treatment complemented with enzymatic hydrolysis promoted a slight improvement on chemical oxygen demand (COD) removal (90% vs. 82%), as compared to the control.

Also, the same group reported enzymatic pre-hydrolysis for anaerobic treatment of poultry slaughterhouse effluents instead of synthetic model wastewater [126]. The effect of *P. restrictum* SEP concentration (0.1–1.0%, w/v) for pre-hydrolysis on the biological treatment was explored. It was observed that COD removal efficiency (85% vs. 53%) and biogas production (175 mL vs. 37 mL) with pre-hydrolysis effluents was much higher as compared to those in the control. In addition, the optimal concentration of SEP was 0.1% (w/v) and pretreatment with higher SEP concentrations (0.5% and 1.0%, w/v) did not afford high COD removal efficiencies. In a recent report, it was also found that 0.1% (w/v) SEP from another source *Penicillium* sp. was the optimal concentration for pre-hydrolysis of slaughterhouse wastewater [127]. Interestingly, the COD removal efficiencies (more than 95%) with no acclimated sludge showed an independent relationship on pre-hydrolysis conditions. However, when the sludge was reused (once or twice) the COD removal efficiencies (69.8% and 53.4%) in the control were considerably lower than those (93.8–98.4%) with pre-hydrolysis. It was found that after the biological treatment there were 19.9% and 8.6% fat adhered to the sludge in the control and pre-hydrolysis effluents, respectively. It might account for the enhanced treatment efficiencies by using enzymatic pre-hydrolysis. Recently, Rosa et al. attempted to explain the different performance of anaerobic systems in the presence and absence of pre-hydrolysis step from the microbial profiles in the sludge [128]. Through PCR-denaturing gradient gel electrophoresis analysis of the *Bacteria* and *Archaea* domains, it was indicated that the observed differences were intrinsically related to the microbial diversity of the anaerobic sludge.

Damasceno et al. proposed that the addition of SEP might be a better solution for operational problems resulted from sudden fat overloads in the activated sludge system [129]. In fact, lower COD and turbidity values in the final effluents and lower fat accumulation in the sludge confirmed the efficiency of SEP during high-fat shock loads. It was worthy of note that adding SEP during fat overloads maintained efficient COD removal for 270 days without any operational problems.

Rigo et al. made a comparison between the noncommercial lipase preparation from *P. restrictum* produced in SSF and commercial Lipolase 100T (immobilized lipase from *T. lanuginosus*, Novozymes) in the hydrolysis of oil and grease in wastewater from the swine meat industry [130]. The optimum conditions of hydrolysis for each enzyme were obtained through statistical analysis methodology. The authors proposed that the noncommercial lipase preparation was more suitable for hydrolysis of meat industry effluents due to higher hydrolytic rates. Furthermore, its cost was low, since it was crude enzymatic preparation produced from agro-industrial residues.

6. Conclusions

The industrial demanding for lipase is growing significantly, in spite of its low proportion in current enzyme market. However, it is well known that one of the main obstacles for its large-scale applications, especially its usage for manufacturing popular products or wastewater treatment, is the relatively high cost. It generally comes from the feedstock cost as well as time-consuming and complex downstream-processing. SSF process might open new opportunities for the lipase production since agro-industrial residues with low cost could be used as the feedstock, which is easily envisaged by numerous related articles cited in this review. In addition, simple and efficient downstream-processing systems should be developed, such as liquid–liquid extract with AOT/isooctane reversed micelles. Mutagenesis and directed evolution working as a valuable tool for improving the properties of the lipases such as thermostability, tolerance toward organic solvents and specificity would remarkably expand lipases' biotechnological applications. Moreover, it might be interesting to explore their novel catalytic activities, namely catalytic promiscuity for modern organic synthesis.

Acknowledgments

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