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Lipases and Their Applications-An Applied Research in West Asia

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Lipases are the enzymes that cleave carboxylic ester bonds and are produced by many living organisms like bacteria, fungi, plants and animals. These are the family of hydrolases. Their action is to hydrolyze triglycerides into monoglycerides, diglycerides, glycerol and fatty acids. As the lipases can perform several reactions like esterification, interesterification and transesterification they are widely used in leather, textile, cosmetic, detergent, fine chemistry, cellulose, pulp, paper, medical, effluent treatment, biodiesel production, food and pharmaceutical industries. These enzymes are low cost and easy to produce because of several sources and new technological developments in purification methods. The present paper discusses the various lipase sources, isolation, characterization and purification methods along with their applications.

Keywords: Biodiesel; cosmetic; lipases; paper and textile.

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1. INTRODUCTION

Lipases (also known as triacylglycerol lipases, EC 3.1.1.3) are the class of enzymes that are soluble in water and they hydrolyze triacylglycerols to glycerols and fatty acids and glycerol. Lipases are one of the major biocatalysts that have the wide range of applications in biotechnology. Lipase is ubiquitously produced; they are isolated and purified from different sources that include bacteria, yeast, fungi, animals and plants [1]. Lipoprotein lipase (LPL), one of the lipase family enzymes hydrolyzes triglycerides, and it includes lipase, hepatic lipase and pancreatic lipase. It is synthesized by most of the tissues and it plays important role in the removal of lipoprotein triglycerides, present in the body [2].

As most of the microbial lipases are extracellular, they can be easily produced with high yields and have the wide range of industrial applications [3]. They also play vital role in the synthesis of esters from long chain fatty acids & glycerol and also serve as a biocatalyst in processes like acidolysis, alcoholysis, aminolysis and esterification. The mechanism of lipases is shown below;

Fats (oils) + Water → fatty acid + Glycerol.

Efforts were made to improve the stability of lipases in organic solvents so that they can be used for various applications. Lipase-catalyzed processes are cost effective when compared to traditional processing that has major input requirements and formation of toxic substances as byproducts [4].

[5] had isolated an alkaline thermo-stable lipase producing bacteria from the soil in Isfahan province (Iran). Lipase was extracted using ammonium sulfate precipitation method and purified using ion exchange chromatography. The activity of the purified lipase was found to be 10.2 folds and its molecular weight was 35 KDa.

Lipase produced from *Microbacterium sp*. was isolated from pulp & paper mill using 16S rDNA sequencing method. It was purified by Sephadex G-100 gel column chromatography. The molecular weight was found to be 40 kDa on SDS-PAGE with 20.8% final yield, and activity of the purified lipase was found to be 2.1 folds. An optimal condition for lipase activity was at 50ºC and 8.5 pH. The Km and Vmax values for the purified enzyme were 3.2 mM and 50 µmol/min/mg. The activity of lipase was stimulated by SDS & Triton X-100 and inhibited by Tween 20 & Tween 80 [6].

2. THE ACTIVITY OF LIPASE AT DIFFERENT ZONES

Rhizopus oryzae RO-1 strain was used for the production of lipase using different substrates through solid-state fermentation. The lipase extracted was used for Biodiesel production using different oils as substrate. *Rhizopus oryzae* RO-1 showed maximum lipase production and was selected for further use. *R. oryzae* RO-1 showed increased lipase activity when olive oil was used as a supplement to the substrate. Amberlite-immobilized lipase was used for biodiesel production. High levels of polyunsaturated fatty acids were present in Biodiesel that was produced from algal oil, which made it highly suitable as winter-grade biodiesel [7].

The demand for alkaliphilic and thermophilic lipases is increasing because of their applications in several industries. *Geobacillus thermoleovorans* DA2 was isolated from Southern Sinai, Egypt and lipase were produced using fatty restaurant wastes as an inducing substrate. Maximum lipase production was observed with a substrate concentration of 10% (w/v), inoculum size of 4% (v/v) and an agitation rate of 120 rpm after 48 hours of incubation at 60ºC & pH 10. Addition of galactose as a carbon source and ammonium phosphate as nitrogen source (1% and 0.5% w/v) to the medium resulted in enhanced enzyme production. When TA lipase was added to Triton X-it acts as a degreasing agent by decreasing the lipid concentration. It lowered the total lipid content to 2.6% as compared to a sole crude enzyme (8.9%) or kerosene (7.5%). TA lipase can be used as a substitute for the chemical leather process to boost the quality of leather thereby reducing the environmental hazards [8].

Most of the modern laundry detergents contain enzymes such as lipases, amylases and proteases to remove stains that contain lipids, carbohydrates and proteins. Lipases hydrolyze triglycerides (hydrophobic) present in fats and oils to hydrophilic lipids such as free fatty acids, monoglycerides and diglycerides. [9] observed the enzymatic degradation of triglycerides by using MALDI technique. MALDI imaging of

glycerides was done directly from a textile surface that allowed visualizing the enzymatic degradation process.

[10] isolated lipases from *S. variabilis* NGP 3, *S. albogriseolus* NGP 2 and *S. acrimycini* NGP 1 and investigated the process of enzymatic synthesis of fragrance ester from brewery industry effluent. *S. variabilis* NGP 3 showed maximum conversion percentage of ester (48.72%). Qualitative & quantitative evaluation of fragrance test showed that *S. variabilis* NGP micro encapsulation gave better fragrance than exhausted fabric.

3. COSMETIC AND DETERGENT

In the modern world, the market for commercial products such as cosmetics and detergents is increasing as they have wider uses in everyday life. Many enzymes are being used in cosmetics, but lipases play a major role in industrial production (as catalysts) of various speciality active agents, aroma compounds and esters and a minor role in functional cosmetics [11].

Cosmetic products that give pharmaceutical therapeutic benefit because of their biologically active ingredient are known as Cosmeceuticals. These active ingredients can be extracted and purified from natural sources (herbal extracts, botanicals) as well as they can be obtained by the fermentation process. A cosmeceutical ingredient must contain properties like anti-inflammatory, anti-oxidant, anti-wrinkling, skin whitening, photoprotective activity and anti-ageing. Natural bioactive compounds with low toxicity and exceptional therapeutic properties may offer a new insight into the design and development of beneficial and potent cosmetics [12].

Alkaline and thermo-tolerant lipase enzyme were isolated from *Lactobacillus brevis* and immobilized onto modified γ-Fe_3O_4 florisil nanoparticles. The free and IML (immobilized lipase) enzymes were most stable at alkaline pH in the range of 7.0–10.0 at 60℃. When compared to free lipase enzyme, IML is more stable towards metal ions. IML with detergent (72% & 45%) effectively removed oil stain from cotton cloths when compared to the detergent alone [13].

The 4-hydroxybenzyl acetate obtained by the action of immobilized lipases of *Lactobacillus plantarum* have wider applications in cosmetic industry [14].

4. CHEMISTRY, PHARMACEUTICAL AND MEDICAL

Lipases have the property of performing reactions in both the aqueous and organic solvents. An organic solvent improves the solubility of substrate & reactant in the reaction mixture and allows back word reaction. It is also very easy to recover the product in organic phase in two-phase equilibrium systems hence lipase is used in esterification reactions as is active in organic solvents. The use of organic solvent tolerant lipase has exhibited many advantages such as its ability to shift the reaction equilibrium, higher solubility of the substrate, increased activity & stability, ease of products recovery and regiospecificity & stereoselectivity [15].

Monoacylglycerols and diacylglycerols that are derived from castor oil are useful in the development of lubricants and emulsifiers for pharmaceutical, food use and cosmetics. Similar functions are performed by acylglycerols which can be obtained from the hydrogenation of castor oil. [16] investigated immobilized lipases to generate acylglycerols. Organic solvents were used to modulate the action of lipase to produce mono acylglycerols and diacylglycerols from castor oil. The presence of alkylated oxygen in the solvent is an important factor in supporting lipase activity.

Biocatalysis can also be used as an alternative to chemical processes for the production of single-isomer chiral drugs are one of the most used enzymes. Lipases are used in the synthesis of enantiomerically pure intermediates; this is because of its characteristics like enantioselectivity, regioselectivity and chemoselectivity. Moreover, these enzymes have greater stability in the organic solvents which facilitates modification of the solubility of the organic substrate [17].

Immobilisation methods like entrapment, adsorption and cross-linking or covalent coupling are used to prepare solid state lipase. Some of the practical applications of lipases in organic media include acylation of bioactive compounds & carbohydrates, modification of phospholipids & triacylglycerols, ester synthesis, fatty acid enrichment, enantiomer resolution and biodiesel production [18].

In lipid-water interphase lipase hydrolyse fats and they undergo many biotransformations in micro aqueous conditions. Fungal and yeast lipases catalyze many stereoselective reactions. Based on the oxyanion hole these lipases are categorized into three classes: GX, GGGX and Y. It was observed that that GGGX and Y family are less diverse than GX family. The role of lipases in yeast physiology related to pathogenesis, colonization, biofilm formation and adhesion was investigated [19].

Lipases catalyze the hydrolysis of long-chain triglycerides as they are water-soluble enzymes. They play a vital role in the pharmaceutical, food, chemical and detergent industries. Because of their stability under different physical and chemical conditions and substrate specificity, fungal lipases have many applications in the industries. Most of these enzymes are extracellular and can be easily extracted because of which the cost of production decreases. Lipases are secreted by fungal species to degrade fats and oils present in the soil and reduce soil pollution [20].

5. UTILITY OF ESTERIFIED LIPASE

LipC12, a metagenomic lipase was investigated in the synthesis of ethyl-oleate, a model ester for biodiesel studies. His-tagged LipC12 was purified on a nickel column and immobilized by adsorption on accrual MP-1000. When immobilized on Immunobead 150 (95% in 4 h) LipC12 gave a better conversion of oleic acid than when immobilized on Accurel MP-1000 (80% in 6 h) [21].

Lipase extracted from *Aspergillus niger* was used along with dimethyl sulphoxide (DMSO) and phosphate buffer with acetic anhydride as acetyl donor in the acetylation of nanofibrillated cellulose (NFC). Higher yields of hydrophobicity with the contact angle of 84 ± 9º was observed in enzymatic acetylation in which acyl-enzyme complex was attached on NFC. Lower yields of hydrophobicity with a contact angle of 33 ± 3º was observed in the chemical acetylation with comparable ester content [22].

Lipase obtained from *Pseudomonas fluorescens* was freeze-dried using sucrose so that the environment around the enzyme is more hydrophilic in organic solvents and was tested for the trans-esterification activity and stability. It was observed that five mM Tris-HCl buffer (pH 9.0) with 1% (w/v) sucrose to be optimal for preparation of the lipase by freeze-drying using sucrose. Lipase retained a higher proportion of its activity after incubation for one day at 30ºC in 100% of 1-propanol, n-decane, 1-pentanol, 1-octanol or n-hexane [23].

6. EFFLUENT TREATMENT AND BIODIESEL PRODUCTION

[24] used a laboratory bioelectrochemical system to investigate the efficacy of electrostimulation on COD removal and bacterial lipase activity. When a current of 1 mA and 1100 mg L^{-1} COD concentration was used, maximum activity of the enzyme was observed (38 Umol mL^{-1}). It was reported that suitably applied current could stimulate and improve the activity lipase thereby increasing the efficiency to remove the dissolved oils.

[25] investigated covalent immobilization of lipase obtained from *Candida rugosa* on modified multiwall carbon nanotubes (MW-CNTs) and their application in oily wastewater treatment. The activity of enzyme immobilized with MWCNT increased by five times when compared with lyophilized enzyme thereby enhancing the degradation of waste water containing oils.

Hydrolysis of industrial effluent collected from a fried potato industry was investigated by the lipases obtained from *Burkholderia cepacia* strain ATCC 25416. At the 37ºC temperature and pH 8.0, the enzyme showed maximum activity. The variable that mostly influenced the hydrolysis of agro-industrial effluent was pH, followed by the concentration of enzyme and volume of gum Arabic. This has most important application in potato chip processing industries to reduce their impact on the environment [26].

Biodiesels are methyl esters of fatty acids produced by base-catalyzed transesterification of triacylglycerols with methanol. Lipases are inactivated by the high concentrations of methanol, but some of them are very effective in biodiesel production over traditional acid or base catalyzed transesterification. *Proteus mirabilis* lipase expresses very well in *E. coli* and produces high yields of methyl esters even in the presence of large amounts of water. Dieselzyme 4, a *Proteus mirabilis* lipase variant has greater thermal stability, increased methanol tolerance and improved longevity over wild-type. Immobilised Dieselzyme 4 can be re-used for further biodiesel synthesis [27].

Table 1. Different lipases isolated from various living organisms

Source	Optimum pH	Optimum temp. (ºC)	Molecular weight (kDa)	Km (mM)	Vmax	Specific activity (units/mg protein)	Purification factor (folds)	Reference
Staphylococcus epidermidis strain L2	7.5	40	28	NA	NA	123.95	18.5	[35]
Pseudomonas Sp. ADT3	3.5 and 8.5	22	13.9	0.260	144.93 U/mg/min	527.8	2.9	[36]
Microbacterium sp.	8.5	50	40	3.2	50 µmol/min/mg	2.3 (crude)	2.1	[6]
Bacillus methylotrophic PS3	7.0	55	31.40	NA	NA	693 IU/mg	2.90	[37]
Pseudomonas aeruginosa SRT 9	6.9	55	29	1.11	0.05 mmol/L/min	12307.8 U/mg	98	[38]
Bacillus sonorensis 4R	9.0	80	21.87	NA	NA	NA	12.15	[39]
Pseudomonas putida 922	10	30	45	NA	NA	24 U/ml	5.8	[40]
Bacillus licheniformis	9.0	60	22	NA	NA	0.49 U/mg	1.7	[41]
Pseudomonas sp. strain BUP6	6.9	37	35	NA	NA	96.15 U/mL		[42]
Anoxybacillus flavithermus	9.0	50	64	0.084	500 U/mg	NA	7.4	[43]
Aeribacillus sp. SSL096201	8.0	70	NA	NA	NA	NA	NA	[44]
Bacillus atrophaeus FSHM2	9.0	80	NA	NA	NA	12.48 U mg–1	7.21	[45]
Aspergillus japonicas	7.3	40	40	0.64 × 10–3 mmol	0.25 µmol min–1 ml–1	36.83 U mg–1	14.73	[46]
S. maltophilia CGMCC 4254 (SML)	NA	35	NA	NA	NA	38.9 U/mg	60.5	[47]
Spirulina platensis (Arthrospira)	6.5	45	45	0.02 mM	38.9 µmol min–1 mg–1	NA	375	[48]
Pseudomonas aeruginosa LX1	7.0	40	56	NA	NA	NA	4.3	[49]

Source	Optimum pH	Optimum temp. (ºC)	Molecular weight (kDa)	Km (mM)	Vmax	Specific activity (units/mg protein)	Purification factor (folds)	Reference
Penicillium sp. DS-39 (DSM 23773)	5.5	45	43	NA	NA	308.73 IU/mg	129	[50]
Yarrowia lipolytica NCIM 3639	5.0	25	400	NA	NA	NA	NA	[51]
Geobacillus thermodenitrificans IBRL-nra	7.0	65	30	NA	NA	NA	34	[52]
Pseudomonas stutzeri LC2-8	8.0	30	32	NA	NA	NA	10	[53]

Fossil fuels are depleting fastly and there is a need for research and development of biofuels to power the earth in the future. Lipase extracted from *Burkholderia cepacia* was immobilized and was used for the production of biodiesel from *Jatropha curcas* L. oil (crude). The immobilized lipase acts as an ecofriendly biocatalyst. It is stable and after six cycles of reuse also it retained 73% relative transesterification activity [28].

[29] reported that the combination of immobilized lipase and free lipase catalyzes effective biodiesel production. However, to remove the remaining traces of glycerol from the crude biodiesel product some additional process like water washing is required. Lipase-catalyzed transesterification reaction between glycerol and dimethyl carbonate (DMC) helps in on-line removal of glycerol.

To synthesis biodiesel from palm oil in one-time addition of methanol and solvent-free medium using CBD fused with C-terminal of. Lipase obtained from *G. stearothermophilus* (GSlip-CBD) was immobilized onto magnetic cellulose nanosphere (MCN) and was used in the production of biodiesel from palm oil. Galip-CBD-MCN is a potential biocatalyst for biodiesel production with higher yield and can be reused in one step addition of methanol [30].

7. FOOD AND OTHERS

Microbial lipases have broad substrate specificity because of which they are widely used in various industrial applications like oil manufacturing, organic synthesis, food processing and detergent formulation. Lipase obtained from *Lactobacillus plantarum* was immobilized and its application in meat degradation was reported. It is used in medical field and also in short chains fatty acid esters syntheses like triazole ester and 2,3,4-hydroxybenzyl acetates that have application in flavour industry [14].

Biocatalysts like enzymes (microbial lipases) that are used in the food industry are ecofriendly and are easily extracted. In biotechnology and food industry microbial lipases, especially extremophiles, have a wider range of applications. Thermo-halophile GDSL lipase-encoding gene obtained from *Rhodothermus marinus* RD was cloned and expressed in *E. coli*. This biocatalyst has greater potential in the food industry; it can be used to improve the aroma of foods and lipid processing [31].

Candida rugosa lipase (CRL) is an important biocatalyst because of its remarkable efficiency in both synthesis & hydrolysis. It was reported that the yields of *Candida rugosa* lipase (CRL) and hydroxyapatite-*Candida rugosa* lipase (HAP-CRL) in the synthesis of the aroma ester methyl acetate in hexane were 2.6 and 52.5% respectively. CRL, industrially prominent biocatalyst. HAP is a biocompatible, environmentally suitable carrier that is combined in the preparation of immobilized lipase. The obtained CRL preparation has excellent potential in food and flavour industries [32].

Lipase of *Fusarium solani* NAN103 is overexpressed in *Pichia pastoris* using inducible expression system under the control of alcohol oxidase one promoter (pAOX1) and using constitutive expression system under the control of glyceraldehyde-3-phosphate dehydrogenase promoter (pGAP). When *P. pastoris* was cultivated in wastewater with 1% w/v palm oil under pGAP, the lipase that was produced by the organism degraded 87% of the oil within 72 hours [33].

Aspergillus niger lipase was used in the hydrolysis of food waste to achieve high biomethane production. Two different methods of lipase additions were investigated; first method (method 1), before anaerobic digestion, to pre-treat food waste to pre-decompose lipid to fatty acids; second method (method 2) to add lipase directly to an anaerobic digester to degrade lipid inside digester. Method 1 showed higher biomethane production than method 2. This might be helpful in mitigating environmental impact associated with food waste by providing an alternative for efficient biomethane production from food waste [34].

8. CONCLUSION

Lipases are ubiquitous proteins that have wide hydrolytic action and have proved the versatility in solving several problems. The gene responsible for producing lipases was reported by several researchers and also the advancements in genetic engineering and protein designing to meet the industrial requirements. Various type of lipases extracted from different organisms are showed a variegated response at different zones with some technological aspects and biological purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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