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Production, Purification, Characterization, Stability and Application of Lipase: A Review

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Abstract

Lipases (EC 3.1.1.3) are versatile hydrolytic enzymes that catalyses the breakdown and modification of lipids and have gained considerable importance as industrial biocatalysts. The present review summarizes the major aspects of lipase production, purification, characterization, stability, and industrial applications, with particular emphasis on microbial lipases. The important sources of this enzyme include several microorganisms including bacteria, fungi, and yeasts, because of their wide distribution, ease of cultivation, high productivity, and ability to produce stable enzymes with diverse catalytic properties. The importance of thermostability for improving enzyme performance under industrial conditions is also discussed. Lipases have extensively used in several industries including oleochemical, detergent, food, biodiesel, pharmaceutical, pulp and paper, cosmetic, biosensor, biodegradable polymer, and waste-treatment. Advances in enzyme engineering, immobilization, and bioprocess technology are expected to further improve lipase efficiency and expand their industrial applications.

Keywords: *Lipase, glycerol ester hydrolase, enzyme, application of lipase.*

Introduction

Lipases (glycerol ester hydrolase; EC 3.1.1.3) belongs to the group of hydrolytic enzymesas it can catalyze the hydrolysis of long chain triglycerides [1]. Eijkman isolated the first lipases in 1901 using *Bacillus prodigiosus*, *Bacillus pyocyaneus*, and *Bacillus fluorescens*, which are at currently known as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* respectively [2]. Recent research shows that lipases can be produced by a variety of organisms, including animals, plants, and microorganisms [3]. It has the ability to perform specific chemical transformations and has high potential for commercial applications indifferent sectors, including food processing, textiles, detergents, pharmaceuticals, cosmetics, and the chemical industry [4]. It has been observed that lipases derived from microbes, and specially of bacterial origin have higher rate of commercialization as compared to others [5]. The most used and important bacterial genera which are used for lipase production includes *Bacillus, Pseudomonas*, and *Burkholderia*. The majority of bacterial lipases are extracellular in nature and are made through submerged fermentation. Along with some more contemporary techniques such reverse micellar and aqueous two-phase systems, hydrophobic interaction chromatography is the method that is most frequently used to purify enzymes. Although alkaline bacterial lipases are more prevalent, most lipases can function in a wide variety of temperature and pH conditions [6].

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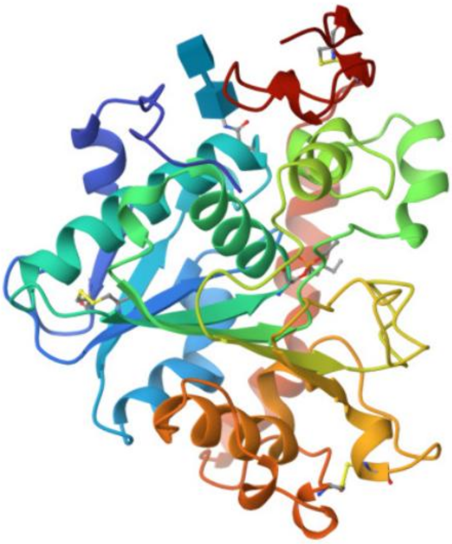

Fig 1. Structure of Lipase (Source: https://www.rcsb.org/structure/1LBS)

Microorganisms from diverse and extreme environments are valuable sources of lipases with distinctive catalytic properties. For example, lipase B from *Candida antarctica* has been successfully displayed in *Pichia pastoris* as a whole-cell biocatalyst [9].

The most common bacterial lipases includes *Achromobacter* sp., *Alcaligenes* sp., *Arthrobactersp.*, *Pseudomonas* sp., *Staphylococcus* sp., and *Chromobacterium* sp [11]. The bacterial strains used for lipase production includes oil industrial wastes, vegetable oil processing factories, dairy plants, paper industries and soil contaminated with oil [12].

Similarly the most common fungal source of lipase includes *Rhizopus, Aspergillus, Penicillium, Mucor, Ashbya, Geotrichum, Beauveria, Humicola, Rhizomucor, Fusarium, Acremonium, Alternaria, Eurotrium, Ophiostoma* [13], [14]. Besides some other commercial fungal lipases includes *Aspergillus niger*, *Candida rugosa*, *Thermomyces lanuginosus*, *Rhizomucor miehei*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus oryzae* and *R. Japonicus* [15].

Lipase derived from yeast source has unique applications in the field of chemical, pharmaceutical and biodiesel industries [16]. *Candida utilis*, *C. rugosa, Rhodotorula* sp., *Yerrowia* sp. and *Pichia* sp. are the most common sources of yeast sources [12].

Table: 1- Different sources of potential microorganisms that act as lipase producers [17]

Bacterial Source	**Fungal Source**	**Yeast Source**
Bacillus stearothermophilus	*Rhizopus chinensis*	*Williopsis californica*
Burkholderia cepacia	*Aspergillus sp.*	*Saccharomyces cerevisiae*
Burkholderia multivorans	*Rhizopus homothallicus*	*Aureobasidium pullulans*
Serratia rubidaea	*Penicillium citrinum*	*Yarrowia lipolytica*
Bacillus sp.	*Penicillium restrictum*	*Rhodotorulam ucilaginosa*

Production of lipase

Lipases, particularly microbial lipases, are industrially important biocatalysts; thus, screening, production, and purification of lipase enzyme from microbial strains are constantly emerging to meet the needs of the pharmaceutical and food industries [12]. Fermentation is the most common process employed for production of lipase. It includes different modes and types of fermentation like batch, continuous and fed batch or submerged fermentation and solid-state fermentation are used depending upon the targeted product (Fig 2) [6], [12].

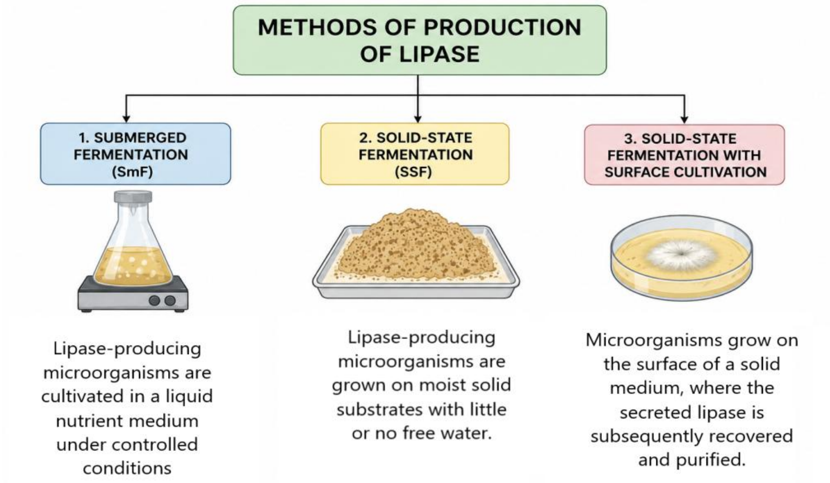

Fig 2: Different methods of production of Lipase

Purification and kinetic characterization of lipases

Depending on whether a microbial lipase is extracellular or intracellular, different purification processes are required. In either scenario, generated proteins are further salted out using ammonium sulphate precipitation procedures [12]. The precipitated protein is also subjected to chromatography and dialysis procedures. The method of choice for chromatography, which is mostly used to purify lipases, varies depending on the size of the proteins and the source of the microorganisms. Finally, theacquiredcrudefractionsaresubjectedto variousenzyme assay protocols and further separation methods such as ion exchange, DEAE-sepharose, and gel filtration chromatography [12].

Thermostability of lipase

For every 10˚C increase in temperature, a reaction's rate nearly doubles. The productivity of the reaction can be considerably increased by running at a reasonably high temperature, presuming the enzyme is stable at high temperatures. Consequently, thermal stability is one of the desirable property of lipase enzyme [18]. Thermostable lipases have been discovered in a wide range of organisms, including *P. fluorescens*, *Bacillus* species, *Bacillus coagulans*, *B. cereus* and *B. stearothermophilus* [19]

Applications of lipases

Use of Lipases can be observed in a wide range of industries and biosynthesis procedures (Fig 3). Some important applications are briefly given below,

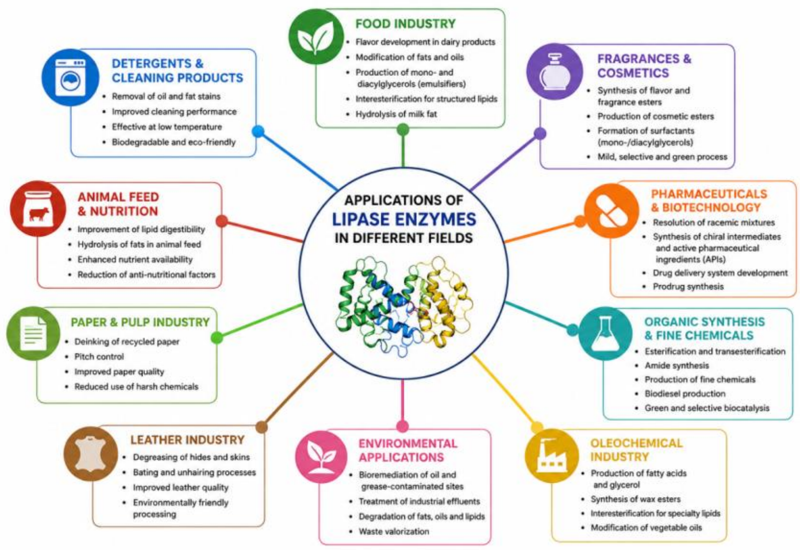

Fig 3. Applications of lipase enzymes in various industries and sectors

Lipases in fat and oleo-chemical industry

Lipases shows wide ranges of reactions and have several uses in the oleochemical sector [20]. In recent times, the trend of using immobilised lipase in the oleo chemistry sector for several reactions, including hydrolysis, alcoholic, and glycolysis using mixed substrates has been proved to be beneficial [4]. Foods contain vitalcomponents like fats and oils. By moving or replacing one or more ofthe existing fattyacid chains in the glyceride, lipases enable to change the characteristics of lipids. In this manner, a relatively cheap and undesirable lipid can be changed into a valuable fat. Among the lipolytic conversions of oils and fats, esterification and interesterification are used to produce value-added products such as specialty fats and partialglycerides using positionaland fatty acid specific lipases, and have a higher industrial potential than fatty acid production in bulk via hydrolysis [19].

Lipases in detergent industry

Following the commercial success of proteases as detergent additives, the enzyme industry made significant efforts to incorporate lipases as a second group of detergent enzymes. Furthermore, the compatibility of lipase was evaluated by comparing it to several commercially available detergents and bleaching agents used to remove fat. Enzymes can be found in all detergent formulations that are currently popular in developing countries. Laundry detergent is becoming increasingly popular as it imparts softness to fabrics, antistatic properties, and is water-soluble. There are numerous laundry detergent brands on the market that claim various qualities of laundry detergent [21], [4].

Lipases in production of biodegradable polymer

Lipases have become important groups of enzymes inorganic synthesis due to their wide range of applications. Lipases, as a biocatalyst, aid in the production of biodegradable compounds. Recently, it was reported that 1-Butyllocate was produced by direct esterification of butanol, and oleic acid tends to decrease the viscosity of biodiesel during the winter. As lubricants, trimethylol-propane esters were also synthesized in the same way. In the presence of organic solvent systems, lipases can also catalyse ester synthesis and transesterification reactions. As a result, there is an opportunity for biocatalyzed polyester production. Lipase biocatalysts can also synthesise aromatic polyesters [22].

Lipases in biodiesel

During the past few years, there has been a lot of interest in biodiesel, which is produced from triglycerides or free fatty acids by transesterification or esterification with short chain alcohols. It has proved as a renewable, biodegradable, and non-toxic fuel over the last few years [23]. Since it provides large conversions of triglycerides to their corresponding methyl esters in briefreaction durations, chemical transesterification has enabled the industrialisation of biodiesel production in many nations. However, the process has a number of drawbacks, it requires a lot ofenergy, is difficult to recover the glycerol, requires treatment of the alkaline waste water, requires removing the acidic or alkaline catalyst fromthe product, and interferes with the reaction with freefatty acids and water [24]. Enzymatic methods for making biodiesel have gained a lot of attention recently since they are more advantageous than chemical ones in terms of reaction circumstances, alcohol to oil ratio, ease of product recovery, and environmental friendliness [22], [26]. Most studies on enzymatic methods for making biodiesel concentrate proves that immobilised extracellular lipases are better than free lipases because they are frequently more stable than free lipases and can be utilized repeatedly without requiring laborious separation. It was discovered that lipases from microbes such *Mucor miehei, Rhizopus oryzae, Candida antarctica*, and *Pseudomonas cepacia* are ideal for the manufacture of biodiesel [27], [29].

Lipases applications in medical and pharmaceutical industries

Lipases have important applications in biocatalysis and can be used in the synthesis of pharmaceutical intermediates and other value-added products, including the production of cocoa butter replacements, human milk-fat substitutes for infant nutrition, cosmetic esters such as isopropyl myristate, monoglyceride emulsifiers, flavour compounds such as γ-decalactone, and the stereoselective synthesis or resolution of chiral pharmaceuticals such as naproxen, ibuprofen and ACE inhibitors [19].

Lipases application in pulp and paper industry

In the production of pulp and paper, "pitch," or the hydrophobic elements of wood (mostly triglycerides and waxes), presents significant challenges. Pitch is eliminated by lipases from the pulp used to make paper. Japan's Nippon Paper industries has created a pitch control technique that hydrolyzes up to 90% of the triglycerides in wood using the fungal lipase *Candida rugosa* [30].

Lipases as biosensors

The estimate of lipids in a patient's analytical sample in order to diagnose cardiovascular complaints is a crucial use of microbial lipases. The fundamental idea behind quantitative estimation is that triacylglycerol, which is present in the analytical sample, is hydrolyzed by lipase into glycerol and free fatty acids. Then, using an enzymatic and chemicalapproach, the amount of liberated glycerolor free fattyacids is calculated. This makes it possible to gauge the patient sample's lipid content. A biosensor systemusing *C. rugosa* lipase has been created. Because of its high specific activity, *C. rugosa* lipase is non-specific and enables quick liberation of glycerol and fatty acids from lipid [31].

Lipases in cosmetics and perfumery

Lipases have the capability of being used in fragrances and cosmetics because they engage in surfactant activities and fragrance creation through esterification. Monoacylglycerol and diacylglycerols are created using lipases, which are surfactants used in perfume and cosmetics industries. Immature bacteria act as a biocatalyst. It made use of *Rhizomucor meihei* lipase. The business asserts that utilising the enzyme as opposed to the conventional acid catalyst resulting in significantly better products that minimum downstream refinement is necessary [19], [31].

Lipases in food processing industry

Lipases include a wide range of applications in the food processing sector. For instance, lipases in the processing of vegetable oils enable a large increase in oil yield while also improving the aesthetic of the final product. Lipases are added to bread and dairy products to improve and hasten the emergence of fragrant notes. In order to improve the emulsifying abilities of egg yolk lipids, lipases are highly valued in the food business. Additionally, lipases are being researched to produce new functional components and meals, such as human milk fat equivalents or cocoa butter equivalents [32].

Lipases are employed to digest milk lipids and give cheeses their distinctive tastes. When milk fats are hydrolyzed, free fatty acids are created, which are where the flavour derives from [33].

In baking, lipases and phospholipases can modify lipid components and may be used to improve dough handling and product quality [32]. Lipases can contribute to flavour development and, in combination with other baking enzymes, may improve technological properties of baked products [32].

Lipases application in Waste/effluent/sewage treatment/oil biodegradation

In activated sludge and other aerobic waste treatment systems, where thin layers of fat must be continuously scraped off the surface of aerated tanks to allow oxygen to get through, lipases are utilised to break down the fats. The biodegradation of petroleum hydrocarbons is caused by naturally occurring, cold-adapted microbes that break down these pollutants in icy environments, particularly alpine soils. Fungal species can be used in enzymatic oil processing in industry, coastal environments, and to decompose oil spills [34].

Conclusion

Thus, this review demonstrated the potential of lipases as biocatalysts and its applications in a variety of sectors. Although the market for lipases still represents a small fraction of the overall food enzymes market, it is anticipated to expand with the introduction of new products as well as its applications. In order to increase the usefulness of these enzymes even more, protein engineering and natural selection are producing lipases with improved characteristics. In order to use the lipases efficiently, developments in bioreactor and reaction technologies are to be occurring concurrently.

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