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A review on lipases: sources, estimation and applications

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Abstract
Lipases are ubiquitous enzymes that cleave a variety of reactions and exhibit a broad range of industrial applications. Lipases found in microbial sources such as fungi, bacteria and yeast and are important from commercial point of view. Microbial lipases are considered more useful than animal and plant sources because of their stability and reactions they catalyze. This review provides an overview about different sources and industrial applications of lipases. There are several applications of lipases such as in oleo chemical industry, in detergent industry, as biosensors, in food processing industry for flavor development, sewage treatment and in cosmetics. Today lipases have tremendous demand and because of the reactions they catalyze. They are able to induce esterification and transesterification and hydrolysis reactions.

Keywords: lipases, sources, industrial applications, microbial

Introduction
Lipases (EC 3.1.1.3) are classified as triacylglycerol acyl hydrolases and they do hydrolysis of fats and oils to give fatty acids and glycerol (Singh and Mukhopadhyay, 2012) [1]. Lipases are concerned in various conversion reactions, such as aminolysis, acidolysis, esterification, alcoholysis, transesterification and interesterification (Savitha *et al*., 2007) [2]. The tendency of lipases to execute extremely definite chemical conversion (biotransformation) has made them progressively more popular organic synthesis, in the detergent, cosmetic, food and pharmaceutical companies (Park *et al*., 2005; Grbavcic *et al*., 2007 Gupta *et al*., 2007; Franken *et al*., 2009]) [3, 4, 5, 6].
Extracellular production of lipase has been deliberated for many of fungi, mainly yeasts, zygomycetes and hyphomycetes (Ksandopulo, 1974; Tsujisaka *et al*., 1977; Chander *et al*., 1980; Akhtar *et al*., 1983) [7, 8, 9]. Lipase secretion has also been demonstrated for few coelomycetes and ascomycetes (Oso, 1978; Reddy and Reddy, 1983) [11, 12]. Lipolytic potential also reported in *Lipomyces starkeyi*, *Geotrichum candidum*, *Cunninghamella verticillata*, *Humicola lanuginose*, *Aspergillus* sp., *Pencillium* sp., *Candida rugosa* and *Rhizopus* sp. (Tsujisaka *et al*., 1973; Tahoun *et al*., 1982; Jensen, 1983; Sztajer *et al*., 1988; Sztajer and Maliszewska, 1989 Iizumi *et al*., 1990; Jacobsen *et al*., 1990; Okeke and Okolo, 1990; Petrovic *et al*., 1990; Wu *et al*., 1990; Gopinath *et al*., 2000; Gopinath *et al*., 2002; Gopinath *et al*., 2003; Salihu *et al*., 2011; Thota *et al*., 2012) [13, 14, 15, 16, 17,n 18, 19, 20, 21, 22, 23, 24, 25, 26, 27].

Historical background
In the year 1856, lipase was primarily discovered in pancreatic juice by Claude Bernard. Lipases were demonstrated for the first time in seeds of plants. The animal pancreatic extracts were used conventionally like the source of lipase for the commercial uses. The producers of lipase were extensive in the nature. However, the microbial sources of lipase were explored when the industrial lipase potential heightened and when the need for lipases could not be satisfied by the source of animal sources. The first study about fungal lipases was reported by Ghosh *et al*., (1996) [28]. In the year 1994, Novo Nordisk reported the first commercial recombinant lipase 'Lipolase' that was developed from the *Thermomycesl anugiwnosus* fungus and expressed in *Aspergillus oryzae*. The fungi competent to synthesize lipases are found in numerous habitats, such as dairy by products, seeds and deteriorated food and soils polluted with wastes of vegetable oils (Sharma *et al*., 2001; Ko *et al*., 2005) [29, 30].
Over 300 years ago, enzymes hydrolyzing triglycerides have been studied and synthesis of ester and also capability of catalyze hydrolysis has been demonstrated about 70 years before (Hasan *et al*., 2006; Hossain *et al*., 2010) [31, 32].

Sources of lipases

Microbes, being ubiquitous in allocation, numerous microbes such as molds, yeasts, bacteria and some protozoa are recognized to produce lipases for the degradation of lipid materials (Vadehra, 1974; Macrae, 1983; Ginalska *et al*., 2004; Saeed *et al*., 2005; Anbu *et al*., 2011; Nwuche and Ogbonna, 2011; Nagarajan, 2012; Abrunhosa *et al*., 2013) [33, 34, 35, 36, 37, 38, 39, 40].

Fungi

Most economically significant lypolytic fungi are reported, such as *Rhizomucor* sp., *Penicillium* sp., *Aspergillus* sp. and *Mucor* sp. Lipase production varies among different fungal strains. Lipase production differs from fungus strain to strain, cultivation conditions, temperature, growth media composition, type of organic and inorganic nitrogen sources, pH and incubation time (Cihangir and Sarikaya, 2004) [41]. Colen *et al*., (2006) [42] reported 59 lipolytic fungal strains using improvement culture approaches from Brazilian savanna soil. Kaushik *et al*., (2006) [43] demonstrated lipase production from *Aspergillus carneus* and reported a highest lipase activity of 13 U mL^{-1}.

Bacterial lipases

Among bacterial lipases being oppressed, which are from *Bacillus* demonstrate fascinating properties to facilitate them as a potential aspirant for industrial point of applications. Carvalho *et al*., (2008) [44] reported a bacterial strain from soil (petroleum polluted) and obtained a highest lipase value was 1,675 U mL−1 after 120 h of fermentation. Shariff *et al*., (2007) [45] reported a bacterial strain L2 (thermophillic bacteria) from a hot spring, Malaysia. Through broth and plate assays, an extracellular lipolytic activity was reported at temperature of 70 °C after 28 h of fermentation process.

Yeast lipases

Vakhlu and Kour, (2006) [46] demonstrated that *Candida rugosa*, *Rhodotorula glutinis*, *Candida* deformans, *Candida cylindracea*, *Candida* curvata, *Candida* parapsilopsis, *Yarrowia lipolytica*, *Pichia burtonii*, *Pichia bispora*, *Candida* valida, and *Saccharomycopsis crataegenesis* are main lipase producing sp of yeasts. Kumar and Gupta, (2008) [47] demonstrated fifteen yeasts from oil sludge and petroleum areas in Delphi (India). These isolated yeasts were purified and tested for lipase production ability.

Quantitative strategies

Activity of lipase may be calculated using totally different quantitative strategies like fluorescence, titrimetric, quantitative chemical analysis, and chromatographic methods.

Titrimetric strategies

In these strategies, titrimetric estimation of fatty acids liberated by the chemical change activity of lipases on triacylglycerols is carried out. The substrates used in these strategies containing vegetable oil, tributyrin and triolein. Thymolphthalein or acid-base indicators are used for titrimetric methodology. The frequently used titrant is caustic soda. There are various reports (Bhavani *et al*., 2012; Ghasemi *et al*., 2014; Mukhtar *et al*., 2015; Rai *et al*., 2014; Ravindranath *et al*., 2014) [48, 49, 50, 51, 52] of estimation of enzyme activity through titrimetric methodology. Mukhtar *et al*., (2015) [50] stated quantitative screening of 7 fungal isolates by means of this method. Maximum activity (5.12 U ml-1) was obtained by *A. niger* as compared to alternative isolates.

Colorimetric methods

They are more simple, sensitive and rapid as associated to volumetric methods. Copper soap method is one amongst the quantitative chemical analysis method, within which the fatty acids which are liberated by the chemical change activity of the lipase produce blue colour soaps of metallic element complexes which may then be separated into an organic solvent followed by spectrophotometric estimation (Duncombe, 1964; Lowry and Lowry, 1976) [51, 52]. There are various reports (Namboodiri *et al*., 2000; Prabakaran *et al*., 2009: Veerapagu *et al*., 2013) [53, 54, 55] of estimation of lipase action by copper soap technique.

Colorimetric methods via *p*-nitrophenyl esters

In these strategies, artificial lipidic substrates such as paranitrophenyl esters of the elongated chain fatty acids are used which upon chemical action by lipase, change into yellow coloured product (*p*-nitrophenol). The absorbance of *p*-nitrophenol is decided at 405-410 nm (Becker *et al*. 1997) [56]. The artificial substrates used for lipase analyze are as follows: *p*NP-propionate, *p*NP-caprylate, *p*NP-palmitate, *p*NP-stearate, *p*NP-valerate and *p*NP-laurate. Naphthyl esters may also be used as substrates that upon enzymatic chemical reaction convert into phenol, which forms a red coloured compound with diazonuim salts. The absorbance of red colour compound is measured at 560 nm. Some usually used naphthyl esters were naphthylcaprylate, naphthyl propionate and naphthylacetate (Gandolfi *et al*., 2000) [57].

Lipase assay using *p*NP-caprylate (Pereira *et al*., 2013) [58], *p*NP-acetate (Paranjothi and Sivakumar, 2016) [59] *p*NP-palmitate (Joshi *et al*., 2006; Karanam and Medicherla, 2008; Iftikhar *et al*., 2010; Xia *et al*., 2011; Mahmoud *et al*., 2015) [60, 61, 62, 63, 64] and *p*NP-propionate as substrates has also been established. Abd-Elhakeem *et al*., (2013) [65] described assay of lipase via phenyl acetate as substrate that upon enzymatic chemical reaction converts into phenol and liberated phenol may be determined by folin ciocalteu chemical reagent.

Applications

Lipases are thought to be the third major enzyme cluster, after proteases and carbohydrolases, supported total sales volume (Jaeger *et al*., 1998) [66]. Lipases are associate degree integral an element of the industries starting from dairy industries, detergents, agrochemical, food and pharmaceuticals to tea industries, oleo-chemicals, leather and cosmetics and in numerous bioremediation processes. Lipase also used to enhance the degradation of polyurethane and fatty waste.

Lipases in detergent

The practice of enzymes/lipases in detergents still remnants the one most important marketplace for industrial lipases (Hasan *et al*., 2006) [31]. For the formation of soap powder formulations, *Pseudomonas* enzyme preparations were used. *Pseudomonas alcaligenes* and *Pseudomonas medocina* lipases are industrially made by Genencor international USA, as preservative of detergent.

Lipases produced from fungal strain plays vital role in the removal of oil strain from cotton fabrics and are also an important part of detergent mixtures (Aaslyng *et al*., 1991) [67]. The industry Novo Nordisk exhibited a fungal lipase in 1988, an enzyme which is competent to dislocate fatty stains. *Humicola* strain produces this enzyme naturally but in a small

concentration for commercial use. Through gene cloning method, the lipase coding gene was replaced with gene of *A. oryzae* to enhance its production. Now, this fungus produces lipases in economical significant yields therefore, it can be used in laundry purposes (Hasan *et al*., 2006) [31].

Lipases as Biosensors

Utilization of microbial lipases as biosensors is new developing approach. Biosensors can be electronic or chemical in nature. Biosensors are mainly used to determine lipids for experimental purpose. Non-specific lipase of particularly *C. rugosa* has been chosen to permit rapid release of glycerol. To the bio recognition assembly in DNA, lipase biosensor *C. rugosa* optically conjugates with it. Hence, it developed as probe (Pandey *et al*., 1999) [68].

Lipases in Cosmetics and perfumery

Lipases have concealed application in cosmetics and perfumeries as a consequence of it demonstrate activities in aroma production and in surfactants. Retinoids are of huge commercial potential in pharmaceuticals and cosmetics such as membrane care goods. H_2O soluble retinoids derivatives were contrived by chemical method reaction of immobilized enzyme (Maugard *et al*., 2002) [69].

Lipase in oleo chemical industry

Usage of lipases in oleo chemical industry reduces thermal degradation during acidolysis, glyceroysis, alcocholysis and hydrolysis and also saves energy. Lipases can also catalyzes alcoholysis (wherever an acyl compound is replaced between an alcohol and an acyl glycerol), acidolysis (wherever an acyl compound is replaced between a carboxylic acid and an acyl glycerol) and transesterification (where two acyl compounds are exchanged between two acylglycerols) depending upon the type of substrates (Vulfson, 1994) [70].

Lipases in food processing, improving quality and flavor development

Lipases are now an essential element of the current food processing industry (Theil, 1995) [71]. They are advantageous intended for the development of flavors in cheese and for inter esterification of oils, fats. Cocoa butter having elevated fat value that having stearic acid and palmitic acid which has 37℃ melting point. The accumulation of lipases to the food leads to release the small chain free fatty acids which provide the prickly, tangy flavor while the liberation of average chain fatty acid gives the foamy flavor to the product. For the conversion from one form to another the lipases are used such as from tri-acylglycerol to di and mono- acylglycerol (Vulfson, 1994) [70].

Lipases application in sewage treatment or oil biodegradation

Lipases are used in aerobic waste processes and in oxygen activated sludge processes, where skinny layers of fats is essentially be regularly detached from the exterior of oxygenated tanks to allow oxygen transport into the tank (to continue living surroundings for the biomass). So, that thin fat affluent layer can be digested by lipases. Seven genotypes concerned in the dilapidation of *n*-alkanes (*Rhodococcus* spp. alkB1; *Acinetobacter* spp. alkM; *P. putida* GPo1 alkB; and *Rhodococcus* spp. alkB2), polycyclic aromatic hydrocarbons (*Mycobacterium* sp. strain PYR-1 nidA and *P. putida* ndoB) and aromatic hydrocarbons (*P. putida* xylE), was reported in twelve oil –contaminated soil (Gopinath *et al*., 1998) [72].

Lipases in medical applications

Galleria mellonella (wax moth) secrets extracellular lipase and was found to cause bacteriocidal effect on (MBT) *Mycobacterium tuberculosis* H37Rv. This beginning investigation may be reported as part of universal unselected selection of natural and other resources for investigating novel capable sources of drugs (Annenkov *et al*. 2004) [73]. Iovastain is a drug which reduces serum cholesterol level and lipase used to synthesize it was reported from *Candida rugosa* (Matsumae *et al*. 1993) [74].

Conclusion

Lipases are flexible enzymes that do hydrolysis of oils and fats to give free fatty acids and glycerol. Microbial lipases currently give much value in developing industrial applications. Lipases are also involved in various conversion reactions. Lipases are significant bio-catalysts for elevating important application in diverse industrial applications. Lipases are competent to catalyze new reactions. Therefore lipases are prospective apparatus for the natural chemists. The broader appliance of microbic lipases in the biotechnological field has obligatory the constant development and examination of new lipases with wide substrate acceptance and maximum stability.

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