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Microbial Lipases: Production and Applications: A Review

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Abstract

Enzymes are considered as naturally occurring biocatalysts that have wide range of applications and uses in our daily life. Enzymes can be synthesized artificially having classification of different categories. Lipase is biocatalyst, present naturally in pancreatic juice and stomach. It helps to balance correct gall bladder function. Lipases can be isolated from various species of fungi, bacteria, yeast, animals and plants. These are widely used for biotechnological applications. Lipases isolated from microorganisms are used in numerous industries such as fat and oil industry, pulp and paper industry, textile industry, food industry, cosmetic industry and also in Oleochemistry, environmental management, tea processing, biosensors, diagnostic tools, medical for IBS and for celiac disease, ester and organic synthesis, flavor development of cheese (e.g. italase and capilase) and improving quality. Research on microbial lipases is increasing day by day due to their great commercial potential. Economically, it is reasonable to carryout chemical reactions in the presence of enzymes because they save time and minimize the use of chemicals.

Keywords: Enzyme, biocatalyst, lipase, extraction, applications, industries

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1. Introduction

An Enzyme is a protein molecule which is considered as biological catalyst. Each enzyme reacts only with specific reactant (substrate). Enzyme regulation occurs from higher to lower activity state and vice versa. Enzymes occur naturally and can also be synthesized artificially. The most common naturally occurring enzymes are amylase (metabolizes starch), Cellulase (cellulose of plant, grains, seeds and vegetables), Invertase (sucrose), Lactase (lactose) and Lipase (fats) (Corleone *et al*., 2015). Major examples of enzymes include amylase, lactase, diastase, sucrose, maltase, invertase, glucoamylase, alpha-glycosidase, protease, peptidase and lipase etc. According to functions of enzymes they are classified into six major categories having different EC number (classification based on mechanism of working of enzyme). EC1 Oxidoreductases (catalyze reduction or oxidation reactions), EC2 Transferases (catalyze the shifting of a functional group from one molecule to another), EC3 Hydrolases (undergoes hydrolysis), EC4 Lyases (undergoes generation of double bonds), EC5 Isomerases (catalyze structural changes within a molecule), EC6 Ligases (undergo ligation). Among all of these enzymes, the most significantly used enzyme is Lipase. Before the mid-1980s, lipases were mostly used in laundry applications and in the modification of triglycerides. Advanced research data has proved that they are also very effective biocatalysts to synthesize optically pure compounds for example cyclohexane (Gurung *et al*., 2013). Lipases are basically biological enzymes (triacylglycerol lipase). The hydrolysis of triacylglycerol into fatty acids and glycerol is catalyzed by lipases (Fig. 1). Such a process is known as lipolysis (Svendsen, 2000).

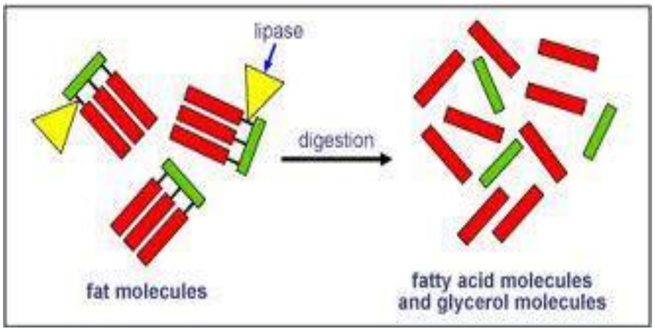

Fig. 1: Working of Lipase

Lipase enzyme naturally occurs in pancreatic juice and stomach. The correct gall bladder function is also maintained by lipases. They also control the volume of fat in body that is synthesized and burned by reduction of adipose tissue (Asian, 2012). Lipases can be purified or extracted from plant, animal, yeast, bacterial and fungal sources sentence needs (Saxena *et al.*, 2003). The characteristic of lipase depends majorly on extraction sources. These properties include specificity, thermo stability (Imamura and Kitaura, 2000), positional specificity (Buchon *et al.*, 2000) and pH etc. (Verma *et al.*, 2012). Lipases catalyze a broad range of reactions known as bioconversion reactions. Esterification, acidolysis, interesterification and amino lysis come under bioconversion reactions. Lipases can act on esters of fatty acids, synthetic triglycerides, and natural oils and many more substrates (Buchon *et al.*, 2000). Lipases distinctly act between the phase of an aqueous and a non-aqueous phase includes glycerol and fatty acids esters can be produced with the help of lipases at the activity stage of low water sentence needs explanation (Fig. 2). Long chain fatty acids and glycerine having emulsified esters can be break down for example triolein and tripalmitin (Aravindan *et al.*, 2007).

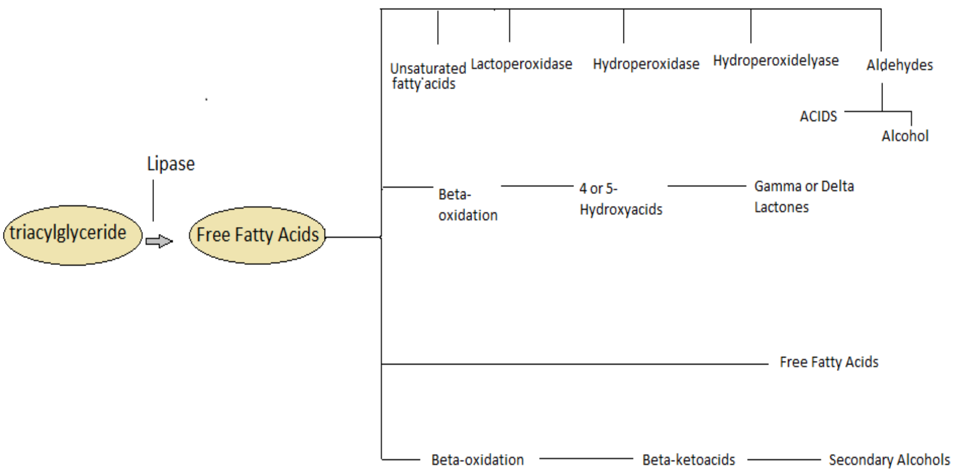

Fig. 2: Catabolism of free fatty acids

The component that animal lipase is made from is properly registered, however, the sources mentioned in literature direct towards drying and grinding of pre-gastric glands at the base of the calves tongues (Benjamin and Pandey, 1998). Recently, a new class of lipases has become commercially available that act on polar lipids as well as triglycerides (Christiansen *et al.*, 2003). These new lipases have a greater activity on galactolipids and phospholipids than on triglycerides. Microbial lipase used in industry, is extracted from yeast, fungi and bacteria. The most preferable source is fungi because fungal enzymes extraction from fermentation media and mostly are extracellular (Ahmed *et al*., 2007). Lipases are widely present in nature, but due to greater stability, wide availability and low production cost, microbial lipases are more significant than lipases extracted from plant and animal sources (Beisson *et al*., 2000).

Distinctive Features of Lipases

Since 1980s, the requirement for lipases has been increased. Their use as an industrial catalyst is increasing day by day due to the favorable properties like high catalytic efficiency, bio-degradability and high specificity (Arife *et al*., 2015). The distinctive features of lipase including temperature (Optimum temperature is 55 °C), specificity, non-toxic nature, pH dependency and activity in organic solvents are major factors that are contributing to lead the demand of lipase in food industry (Verma and Kanwar, 2008). Lipases are investigated for synthetic andhydrolytic having different extraction sources. The utilization of mono-, di-, tri-glycerides and free fatty acids during trans-esterification, high yield or activity in non-aqueous medium, resistance to variation in temperature (Lithauer *et al*., 2002), low product inhibition, less reaction time and pH 8 are most desired features (Kumar *et al*., 2012). Furthermore, under mild conditions of temperature and pH, lipases can undergo reaction. This characteristic of lipases helps to reduce energy demand at infrequent pressures and temperatures to direct reactions. Thus, the destruction of reactants and products that remains unstable during reaction can be protected because it changes the kinetics of the reactions. Lipases can act without co-factor with their substrate and they also show stability in organic solvents. These features are main reason of increasing demand of microbial lipase in biotechnology.

Production of Lipases From Various Microorganisms

Among all the bacterial sources for production of lipases, Bacillus show remarkable properties. All the bacterial lipases are produced at different reaction conditions e.g pH and temperature with respective substrates and synthetic medium (Ertugrul *et al.,* 2007*)*. The production of lipases from fungi also varies depending upon the conditions of synthesis (composition of medium, ph, temperature, sources of carbon and nitrogen) (Cihangir and Sarikaya, 2004). Microorganisms that produce lipases are found in various habitats i.e vegetable oil processing factories, industrial wastes and dairy plants (Sharma *et al*., 2001). Lipase production from yeast also carried out on different conditions for different species (Vakhlu and Kour, 2006; Wang *et al*., 2007).

The production of microbial lipases highly depends upon the composition of medium and carbon sources besides physiochemical factors (pH, temperature) are shown in table 1. The production of lipases generally carried out in the presence of lipid (oil or any inducer i.eglycerols, triglycerols, bile salts, fatty acids, tweens and hydrolysable esters) (Gupta *et al*., 2004; Sharma *et al*., 2001). In order to obtain high yield of lipases, lipid carbon sources are essential. For production optimization and growth of lipases, nitrogen sources and other micronutrients should be selected very carefully. These requirements of nutrients can be fulfilled by agro-industrial residues having necessary components for the development of microorganisms, defined compounds (oils, sugars) and complex compounds (yeast extract, peptone, malt extract) (Rodrigues and Iemma, 2005).

Industrial Applications

Lipases are considered as fundamental part of numerous industries including pharmaceuticals, tea industries, dairy industry, cosmetics, food industry, leather industry, detergents, oleo-chemicals, agrochemicals and of many bioremediation processes. Newer micro-organisms are being selected for the production of lipases having desirable features due to their massive applications in industries (Patil *et al*., 2011).

Table 1: List of lipase producing microorganisms at varying pH and temperature

Microorganism	Type	Temperature	pH	References
Acinetobacter radioresistens	Bacteria	30 °C	5.5	(Zhao *et al.,* 2013)
Pseudomonas sp.	Bacteria	10-70 °C	5.0-10	(Latip *et al.,* 2016)
Pseudomonas aeruginosa	Bacteria	40 °C	7.5	(Bose *et al.,* 2013)
Staphylococcus caseolyticus	Bacteria	37 °C	6-8	Sharma *et al.,* 2014)
Biopetro-4	Bacteria	30 °C	5.0	(Carvalho *et al*., 2008)
Bacillus stearothermophilus	Bacteria	50 °C	8-9	(Ekinci *et al.,* 2015)
Burkholderia cepacia	Bacteria	25 °C	7	(Abdulla *et al.,* 2013)
Burkholderia multivorans	Bacteria	55 °C	8	(Chaiyaso *et al.*, 2012)
Serratia rubidaea	Bacteria	35 °C	8	(Neihaya *et al.*, 2012)
Bacillus sp.	Bacteria	37 °C	7	(Rebeca *et al.,* 2013)
Bacillus coagulans	Bacteria	40 °C	9	(Alkan *et al*., 2007)
Bacillus subtilis	Bacteria	70 °C	8	(Emtenani *et al.,* 2013)
Rhizopus arrhizus	Fungi	40 °C	9	(Wang *et al.*, 2014)
Rhizopus chinensis	Fungi	20-70 °C	8	(Teng *et al*., 2009; Wang *et al*., 2008; Teng and Xu, 2008; Sun and Xu, 2008)
Aspergillus sp.	Fungi	45 °C	4	(Zubiolo*et al* 2014)
Rhizopus homothallicus	Fungi	35-37 °C	6-7.2	(Colla *et al.*, 2015)
Penicillium citrinum	Fungi	35 °C	7	(Kumar *et al.,* 2016)
Penicillium restrictum	Fungi	30 °C	6.5	(Mukhtar *et al.,* 2015)
Penicillium simplicissimum	Fungi	20-70 °C	6-9	(Delgado and Fleuri, 2014)
Penicillium verrucosum	Fungi	30.5 °C	6	(Pinheiro *et al*., 2008; Kempka *et al*., 2008)

Geotrichum sp.	Fungi	45 °C	7.5	(Yan and Yan, 2008; Burkert *et al*., 2004) (Pan *et al*., 2012)
Geotrichum candidum	Fungi	40 °C	7	(Maldonado *et al.*, 2016)
Aspergillus carneus	Fungi	37 °C	7.2	(Colla*et al* 2015)
Rhizopus sp.	Fungi	40 °C	8	(Martinez-Ruiz *et al*., 2008; Khayati*et al* 2013)
Aspergillus niger	Fungi	45 °C	4	(Dutra *et al*., 2008; Mala *et al*., 2008; Falony *et al*., 2006)
Rhizopus oryzae	Fungi	37 °C	7	(Khaskheli *et al*., 2013)
Colletotrichum gloesporioides	Fungi	45 °C	4-11	(Sande *et al*., 2015)
Candida utilis	Fungi	30 °C	4	(Qureshi *et al*., 2013)
Candida rugosa	Fungi	50 °C	10	(Rajendran *et al*., 2008; Boareto *et al*., 2007; Puthli *et al*., 2006; Zhao *et al*., 2008)
Candida cylindracea	Fungi	50 °C	7-8	(Kim and Hou, 2006; Hofi *et al*., 2011; He and Tan, 2006)
Rhodotorula mucilaginosa	Yeast	30 °C	5	(Nuylert *et al*., 2013)
Yarrowia lipolytica	Yeast	30-50 °C	3-8	(Lopes *et al*., 2009; Alonso *et al*., 2005; Kar*et al*., 2008; Ficker*set al*., 2006; Amaral*et al*., 2007; Dominguez *et al*., 2003)
Aureobasidium pullulans	Yeast	35 °C	7	(Liu *et al*., 2008)
Saccharomyces cerevisiae	Yeast	55 °C	4-10	(Varthini *et al*., 2014)
Williopsis californica	Yeast	27 °C	7	(Thakur *et al.*, 2012)
Rhodotorula mucilaginosa	Yeast	37 °C	6	(Luciana *et al.*, 2016)

Fat and oil processing

In food processing industry, the modification of oil and fat is one of the most important areas (Gupta *et al*., 2003). Fats and oils are essential constituents of foods. The properties of lipids can be transformed by lipases when the position of fatty acid is changed in glycerides and also by exchanging one or more than one fatty acids with new ones. Thus an inexpensive and less needed lipid can be converted into greater value fat. By the use of highly selective phospholipases, phospholipids in vegetable oils can be eliminated. This process is latest developed and non-toxic to environment (Clausen, 2001).

Food industry

There are vast applications of lipases in the field of biotechnology by which many biotech goods are produced and used at homes. To improve the characteristics of food stuff, the consumption of enzymes is done by food science solicitations. Although lipases have many applications in food industry but mostly they are used in flavor development and cheese ripening. The use of lipases is *ex situ* to obtain the food stuff having high nutrients, modification of structure by Tran- or inter-esterification and to develop flavor (Reetz, 2002). To enhance flavor in food stuff, the production of esters of fatty acids bearing short chain and alcohols is done. These are mostly used flavor compounds. Thus, to maintain the life time of food and to enhance the flavor, lipases are most important and widely used biocatalyst (Macedo *et al*., 2003).

Detergents

In developed countries detergent making with the help of enzymes is common now and in detergents enzymes used greater than 50% as ingredient. The detergents which are used in laundry has become more popular due to the wide use in washing machine, resiliency to fabrics, softness producers and anti-staticness. Fabrics quality and texture can retain with lesser wash temperature which is also energy saver is modern method in detergent industry (Weerasooriya and Kumarasinghe, 2012). In term of volume and value enzymes mainly consumes in detergent industries. The detergent is eco-friendly with the use of enzymes to avoid the use of harsh strains. Lipase in conjunction with amylase, proteases and cellulases are found in many laundry detergents (Jeon *et al*., 2009).

Oleo-chemical industries

Immobilized lipases used in oleo-chemical industries to initiate the different reactions (alcoholysis, glycerolysis and hydrolyses) used substrates of mix culture. Thus, the high productivity and running process will be nonstop with the help of immobilized enzymes. The immobilized enzymes splitting the fat and economically beneficial because there is no need of large investment for thermal energy equipment. In the oleo-chemical industries lipases applications has great scope becauseit decreases the thermal degradation and save the energy in process of glycerolysis, alcoholysis and hydrolysis (Verma *et al*., 2012). Modifications with enzymes are beneficial and at moderate conditions reaction can be done (Metzger and Bornscheuer, 2006).

Cosmetics and perfumery

Lipases have activities in production of aroma and so has great potential in perfumeries and cosmetics (Metzger and Bornscheuer, 2006). Derivatives (derivatives of what) and vitamin A that is retinoid in pharmaceutical and cosmetics has great applications like the products of skin cares need explanation for the relation of vitamin A with lipase. (Immobilized lipases can be converted into derivatives of retinol water soluble by catalytic reaction) grammatical error (Maugard *et al*., 2002).

Medical applications

Wax moth (*Galleria mellonella*) found the action of bacteria on *Mycobacterium tuberculosis* H3tRv. To detect new sources of medicines this study may be helpful (Annenkov *et al*., 2004). Lovastatin can be produced from lipase isolate from micro-organism *Candida rugosa,* it decrease the serum cholesterol level.

Poly unsaturated fatty acids (PUFAs) obtained by using microbial lipases from plant and animal lipids, like oil of menhaden, borage and tuna oil. A variety of pharmaceutical can be produced by using free poly unsaturated fatty acids and their mono and di-acyl glycerides (Sharma *et al*., 2001). PUFAs, due to their metabolic benefits are used remarkably as pharmaceutical, nutraceuticals and food additives. Immobilized lipases are employing for production of nutraceuticals (Abhijit, 2012).

Baking Industry

Lipolytic enzymes have great use in baking industry. Recently it is suggested that lipases are used to emulsify the supplement or substitute and due to this lipases break the polar wheat lipid into emulsifying lipids *in situ* (Collar *et al*., 2000). Initially the flavor content of the products of bakery was enhanced with the help of lipases through esterification by liberating the short-chain fatty acids. The shelf-life of bakery products can also be enhanced with the help of lipase along with the enhancement of flavors. Through lipase catalyzation the softness and texture can also improves. In baking industry *A. oryzae*is an artificial lipase was used in processing. Lipase, xylanase, amylase and all other hydrolytic enzymes increase the specific volume of breads by reducing the initial firmness (Fariha *et al*., 2006).

Textile Industry

Lipase in conjunction with other enzymes used in desizing in textile industry in this process it removes the adhesive lubricant from the wrap thread that helps the high absorbency levelness in dyeing. In system of denim abrasion the frequency of cracks and streaks is also reduces with the help of lipases. Lipase enzymes and alpha amylase commercially used for desizing of cotton fabrics and denim (Macedo *et al*., 2003).

Flavor development and quality improvement

Recently in food processing industry the modification of fat and oil is one of leading areas it needs green technology and novel economic. Tri-acylglycerols and nutritionally enriched oils of tailored vegetables have great application in future market. To retailoring the oils of vegetables specific fatty acids and specific region of microbial lipases has massive importance. Nutritionally important low calories tri-acylglycerols, oleic acid enriched oil and tri-acylglycerols like substitutes of cocoa butter can be produce with help of low-cost oil. Ester of short chain fatty acids and alcohols can produce with the help of lipases to modify the flavors of food additives and known as fragrance and flavor compounds (Macedo *et al*., 2003). Bio-lipolysis is a process to remove the fat by addition of lipases table 2. In fermentation steps of sausage lipases play a key role and during ripening it helps to measures the changes of long chain fatty acid liberated (Fariha *et al*., 2006).

Table 2: Action of lipases in different industries

Industries	Purposes	Applications
Dairy products	To modify the butter fat, ripening of cheese and hydrolysis of milk	Enhancement of agent of flavor in cheese, butter and milk
Food stuff	To improve quality	Extension of shelf life
Beverages	To improve aroma	Beverages containing alcohol
Health food	To perform trans-esterification	Preparation of healthy food
Laundry/surfactant	To remove strains	Cleaning of clothes
Pharmaceutical	To hydrolyze polyester alcohols	Production of inter-mediates to prepare medicines
Textile	To remove size lubricants	Desizing of cotton fabrics and denim jeans
Cosmetics	Esterification	Sun block and skin care creams, bath oils
Fuel industries	To perform trans-esterification	Production of bio-diesel
Agrochemicals	To done esterification	Production of herbicides
Pollution Control	To hydrolyze oil and grease	Removal of stains

(Kaffarnik *et al*., 2014; Verma *et al*., 2012; Andualema *et al*., 2012; Kobayashi, 2015; Nishat and Rathod, 2015)

CONCLUSION

Enzymes are catalysts that speed up the reaction without taking part in reaction and well known as biocatalysts. Among the natural and synthetic enzymes, the most commonly used enzyme is lipase. The sources by which lipases can be extracted are plants, animals and the easiest one is microorganisms. The production of lipases from different microorganisms is carried out on different physiochemical conditions. Lipases are versatile enzymes having different features depending upon different modes of production. There is a wide and rapid increase of use of lipases for biotechnological applications. Lipases are being used in dairy products, beverage industry, food industry, detergents making, pharmaceuticals, textile industry, cosmetics, fuel industry, fat and oil industry, agrochemicals, pollution control and in production of personal care products. The latest synthetic routes are discovered having upgraded reaction conditions with optimized lipases. By using these advanced lipases different chemicals and pharmaceuticals are being synthesized having better quality. With the help of biotechnology, genetic engineering and protein engineering are playing very important role to modify the features of lipases to increase their applications in all the industries. Lipases also bear some draw backs like high production cost, less commercialization and slow reactivity in some lipase-mediated processes. Due to these factors the use of lipase is restricted to few industries. But in near future these draw backs are going to overcome by new innovative features of lipases that are under study. Many genes of lipase enzymes with unique features are still unknown and need to be explored.

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