Microbial Lipases: Production and Applications: A Review

Shumaila Kiran⁎, Zunaira Arshad, Sofia Nosheenb, Shagufta Kamala, Tahsin Gulzara, Muhammad Sajid Majeeda, Maria Jannata, Muhammad Asim Rafiquec

⁎Department of Applied Chemistry & Biochemistry, Government College University, Faisalabad, Pakistan
bDepartment of Environmental Science, Lahore College for Women University, Lahore, Pakistan
cDepartment of Public Administration, Government College University, Faisalabad, Pakistan

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

Full length article: Received: 12 November, 2016 Revised: 17 December, 2016 Accepted: 18 December, 2016
Corresponding authors: shumaila.asimch@gmail.com

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](image)

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 (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](image)

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 and hydrolytic 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.e.glycerols, 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Type</th>
<th>Temperature</th>
<th>pH</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter radioresistens</td>
<td>Bacteria</td>
<td>30 °C</td>
<td>5.5</td>
<td>(Zhao et al., 2013)</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>Bacteria</td>
<td>10-70 °C</td>
<td>5.0-10</td>
<td>(Latip et al., 2016)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Bacteria</td>
<td>40 °C</td>
<td>7.5</td>
<td>(Bose et al., 2013)</td>
</tr>
<tr>
<td>Staphylococcus caseolyticus</td>
<td>Bacteria</td>
<td>37 °C</td>
<td>6-8</td>
<td>Sharma et al., 2014</td>
</tr>
<tr>
<td>Biopetro-4</td>
<td>Bacteria</td>
<td>30 °C</td>
<td>5.0</td>
<td>(Carvalho et al., 2008)</td>
</tr>
<tr>
<td>Bacillus stearothermophilus</td>
<td>Bacteria</td>
<td>50 °C</td>
<td>8-9</td>
<td>(Ekinci et al., 2015)</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>Bacteria</td>
<td>25 °C</td>
<td>7</td>
<td>(Abdulla et al., 2013)</td>
</tr>
<tr>
<td>Burkholderia multivorans</td>
<td>Bacteria</td>
<td>55 °C</td>
<td>8</td>
<td>(Chaiyaso et al., 2012)</td>
</tr>
<tr>
<td>Serratia rubidaea</td>
<td>Bacteria</td>
<td>35 °C</td>
<td>8</td>
<td>(Neihaya et al., 2012)</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>Bacteria</td>
<td>37 °C</td>
<td>7</td>
<td>(Rebeca et al., 2013)</td>
</tr>
<tr>
<td>Bacillus coagulans</td>
<td>Bacteria</td>
<td>40 °C</td>
<td>9</td>
<td>(Alkan et al., 2007)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Bacteria</td>
<td>70 °C</td>
<td>8</td>
<td>(Emtenani et al., 2013)</td>
</tr>
<tr>
<td>Rhizopus arrhizus</td>
<td>Fungi</td>
<td>40 °C</td>
<td>9</td>
<td>(Wang et al., 2014)</td>
</tr>
<tr>
<td>Rhizopus chinensis</td>
<td>Fungi</td>
<td>20-70 °C</td>
<td>8</td>
<td>(Teng et al., 2009; Wang et al., 2008; Teng and Xu, 2008; Sun and Xu, 2008)</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>Fungi</td>
<td>45 °C</td>
<td>4</td>
<td>(Zubioloet et al 2014)</td>
</tr>
<tr>
<td>Rhizopus homothallicus</td>
<td>Fungi</td>
<td>35-37 °C</td>
<td>6-7.2</td>
<td>(Colla et al., 2015)</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>Fungi</td>
<td>35 °C</td>
<td>7</td>
<td>(Kumar et al., 2016)</td>
</tr>
<tr>
<td>Penicillium restrictum</td>
<td>Fungi</td>
<td>30 °C</td>
<td>6.5</td>
<td>(Mukhtar et al., 2015)</td>
</tr>
<tr>
<td>Penicillium simplicissimum</td>
<td>Fungi</td>
<td>20-70 °C</td>
<td>6-9</td>
<td>(Delgado and Fleuri, 2014)</td>
</tr>
<tr>
<td>Penicillium verrucosum</td>
<td>Fungi</td>
<td>30.5 °C</td>
<td>6</td>
<td>(Pinheiro et al., 2008; Kempka et al., 2008)</td>
</tr>
<tr>
<td>Organism</td>
<td>Type</td>
<td>Temperature</td>
<td>pH</td>
<td>References</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>-----</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Geotrichum sp.</td>
<td>Fungi</td>
<td>45 °C</td>
<td>7.5</td>
<td>(Yan and Yan, 2008; Burkert et al., 2004) (Pan et al., 2012)</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>Fungi</td>
<td>40 °C</td>
<td>7</td>
<td>(Maldonado et al., 2016)</td>
</tr>
<tr>
<td>Aspergillus carneas</td>
<td>Fungi</td>
<td>37 °C</td>
<td>7.2</td>
<td>(Collaet al, 2015)</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>Fungi</td>
<td>40 °C</td>
<td>8</td>
<td>(Martinez-Ruiz et al., 2008; Khayatiet al, 2013)</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Fungi</td>
<td>45 °C</td>
<td>4</td>
<td>(Dutra et al., 2008; Mala et al., 2008; Falony et al., 2006)</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>Fungi</td>
<td>37 °C</td>
<td>7</td>
<td>(Khaskheli et al., 2013)</td>
</tr>
<tr>
<td>Colletotrichum gloesporioides</td>
<td>Fungi</td>
<td>45 °C</td>
<td>4-11</td>
<td>(Sande et al., 2015)</td>
</tr>
<tr>
<td>Candida utilis</td>
<td>Fungi</td>
<td>30 °C</td>
<td>4</td>
<td>(Qureshi et al., 2013)</td>
</tr>
<tr>
<td>Candida rugosa</td>
<td>Fungi</td>
<td>50 °C</td>
<td>10</td>
<td>(Rajendran et al., 2008; Boareto et al., 2007; Puthli et al., 2006; Zhao et al., 2008)</td>
</tr>
<tr>
<td>Candida cylindracea</td>
<td>Fungi</td>
<td>50 °C</td>
<td>7-8</td>
<td>(Kim and Hou, 2006; Hofi et al., 2011; He and Tan, 2006)</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa</td>
<td>Yeast</td>
<td>30 °C</td>
<td>5</td>
<td>(Nyylert et al., 2013)</td>
</tr>
<tr>
<td>Yarrowia lipolytica</td>
<td>Yeast</td>
<td>30-50 °C</td>
<td>3-8</td>
<td>(Lopes et al., 2009; Alonso et al., 2005; Karet et al., 2008; Fickerset et al., 2006; Amaralet al., 2007; Dominguez et al., 2003)</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>Yeast</td>
<td>35 °C</td>
<td>7</td>
<td>(Liu et al., 2008)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Yeast</td>
<td>55 °C</td>
<td>4-10</td>
<td>(Varthini et al., 2014)</td>
</tr>
<tr>
<td>Williopsis californica</td>
<td>Yeast</td>
<td>27 °C</td>
<td>7</td>
<td>(Thakur et al., 2012)</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa</td>
<td>Yeast</td>
<td>37 °C</td>
<td>6</td>
<td>(Luciana et al., 2016)</td>
</tr>
</tbody>
</table>
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 \textit{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 because it 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 (\textit{Galleria mellonella}) found the action of bacteria on \textit{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 \textit{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. oryzaeis* 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

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

(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.
References


