A Review on Applications of Microbial Lipases

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Abstract

Lipases are aqueous soluble enzymes that stimulate the hydrolysis of insoluble substrates (long hydrocarbon chain fat/oil) into their components. Lipases are being extracted from several species of animals, plants, fungi, yeast and bacteria. Microbial lipases are a main category of biotechnologically important enzymes, because of their versatile properties (tolerance to extremes of pH, temperature, metal ions and organic solvents) and easiness of bulk production. Lipases of microbial origin are broadly varied in their enzymatic features and specificity to substrate, which make them especially important for applications in the industries. Amylases and proteases have dominated the world enzyme market but in last few years interest of industries has been shifted towards microbial lipases because of their versatile functions. Their applications in our daily life are rising gradually. Thus lipases are today the enzyme choice for biotechnologist, organic chemist, process engineers, pharmacists, microbiologist, biophysicists and biochemist.

Keywords: *Lipases, substrates, enzyme market, applications, fungi, proteases, oil, hydrolysis, amylases.*

1. INTRODUCTION

Lipases belong to the hydrolase class of enzymes; stimulate the hydrolysis of lipidic substrate (fat/oil) into their monomers (glycerol and free fatty acids) only after absorption to an aqueous-oil interface [1]. Lipases are able to catalyze three kinds of reactions. The catalytic activity of lipases is reversible. They are able to catalyze hydrolysis in a watery environment and esterification in a micro watery environment, in which water content is very low. Depending upon the nature of chemical compound which react with the ester, transesterification is categorized into four subclasses: alcoholysis, acidolysis, aminolysis and interesterification [2], [3]. Lipases are secreted by bacteria [4] yeasts [5] and fungi [6], [7]. In last few years, among the many lipases, microbial lipases are preferred agenda of research due to their diverse applications in industries [8]. About 3000 enzymes have been known till today, however only a certain enzymes are being used in various industries. These are mainly enzymes of hydrolase class (secreted extracellularly), which are capable of degrading natural polymers such as cellulose, lipid, starch, proteins and pectin into their monomers [9]. Following carbohydrases and proteases, lipases are believed to be the third biggest category based on total global sales. They occupy up to 10% enzyme market among the other hydrolases [10]. Lipases found promising application in various industries such as detergent, agrochemical, paper, chemical processing, dairy, pharmaceuticals, oleochemical, cosmetics, polymer synthesis, synthesis of surfactants and personal care products. Several lipases remain functional in organic solvents where they are capable of catalyzing a variety of reactions that comprises transesterification, esterification, regioselective acylation of menthols and glycols and formation of peptides and chemicals [8], [10].

It will not be amazing if lipases acquire the peak position in the area of enzyme application and global enzyme market in the upcoming future. This could be attributed to the quantity of information documented in the literature, ranging from screening, production, strain improvement, optimization, purification, characterization and applications in the industries. Therefore, this review aims to depict the applications of lipases in various industries.

2. REACTIONS OF LIPASES

In addition the classical capability of lipases to stimulate hydrolysis of lipid, they can also stimulate synthetic reactions for eg. esterification and transesterification (acidolysis, alcoholysis, aminolysis and interesterification) in the occurrence of little quantity of water [11]. Divakar and Manohar [12] categorized the lipase catalyzed reactions into three main categories:

(i) Hydrolysis: It happens in watery environment (presence of high quantity of water in the reaction mixture). Ester bonds are cleaved in the hydrolysis reaction. This technique is presently used in the formation of fatty acids, monoglycerides, diglycerides, flavouring substances for dairy products and laundry and domestic detergents.

(ii) Esterification: It happens under little aqueous environment (anhydrous solvents). High quantity of the products (esterified compounds) is achieved under regulated environment. The most common example is the formation of oleic acid esters of primary and secondary aliphatic and terpenic. Other examples are the formation of geranyl esters from the reaction of butyric acid and geranol and menthyl esters from lauric acid and menthol [13].

(iii) Transesterification: It includes the exchange of acid moiety between two or more compounds. According to the type of substrates, lipases are able to catalyze acidolysis (where an ester and carboxylic acid are involved), alcoholysis (where an ester and an alcohol are involved), aminolysis (where an ester is allowed to react with an amine) and interesterification (where two acyl groups are exchanged between two esters) [14].

3. APPLICATIONS OF LIPASES

Lipases play significant role in the industries ranging from pharmaceuticals, food, agrochemical, dairy and detergents to oleo-chemicals, cosmetics, leather, and tea industries and in numerous bioremediation procedures [3], [8].

3.1 Lipases in the detergent industry

Enzymes are frequently used for formulation of detergents in developed countries. Lipases along with the some other enzymes such as amylases, cellulases and proteases are used in detergent industries as they can cleave fats/oils, starch, cotton-fluff and proteins, respectively. Lipases are used as additives in laundry and household (domestic) detergents due to their capability of hydrolysis of fat/lipid. Today, laundry detergents have become popular due to their rising utilization in washing machine, where they provide softness, antistaticness and resiliency to cloths and are easily soluble in water and mild to skins and eyes [15]. Lipases for detergent formulation are selected to fulfill the following requirements: (i) ability to hydrolyze the fat of different compositions (lower specificity to substrate); (ii) ability to tolerate relatively tough conditions (pH 9.0-11.0, 30-60 ºC) of washing; and (iii) ability to tolerate damaging surfactants (linear alkyl benzene sulfonates) and enzymes (proteases), both are chief constituents of several detergent formulations [16]. The first recombinant lipase ‘Lipolase’ was developed by Novo Nordisk (Denmark) in 1994 for commercial use. Genes required for lipase production were isolated from the fungus *Thermomyces lanuginosus* and were cloned and expressed in *A. oryzae*. Similarly, Genenco (USA) also introduced a recombinant lipase ‘Lumafast’ for commercial use.

The primary consumer of enzymes is detergent industries in terms of amount and value. The utilization of enzymes in detergents manufacturing increases the capability of detergents to remove harsh stains and making the detergent safe to the environment. At the present time, mixture of enzymes including cellulases, proteases, lipases and amylases are used in many products of laundary detergent [17].

3.2 Lipases in food industry

Oils and fats are chief ingredients of foods. The physical and nutritional properties of a triglyceride are significantly affected by the location of the fatty acids in the backbone of glycerol molecule, length of the fatty acid chain and its amount of unsaturation. Lipases have the ability to alter the features of lipids by changing the position of fatty acid chains in the triglycerides and substituting one or more existing fatty acids with new ones. In this way, a comparatively cheap and less attractive lipid can be converted to a superior value fat [18]. Lipases are able to stimulate the esterification, hydrolysis and interesterification of oils/fats. Amongst the different lipolytic conversion of fats and oils, interesterification and esterification are utilized to get desired products of higher value [10]. In the recently developed technique, highly selected microbial phospholipases are used to remove phospholipids present in vegetable oils (de-gumming) [19].

3.3 Lipases in flavour development

Lipases are used in many processes of food industries such as in flavor development, ripening of cheese and EMC (Enzyme Modified Cheeses) technology. EMC is a cheese that is incubated along with enzymes at high temperature to synthesize a strong flavour for exploit as a constituent in other products (soups, dressings, dips, snacks, sauses, etc.). Lipases for cheese manufacturing industry are extracted from *A. niger*, *M. miehei* and *A. oryzae* [10]. Lipases are utilized *ex situ* to develop flavours, and to change the structure of fats or oils by inter- or transesterification, in order to get products of improved dietary quality [20].

Flavour substances for eg. S-methyl butanethioate and S-methyl 3-methyl butanethioate are main ingredients of the dairy fragrances, mainly cheese fragrance and of fruit fragrances like banana and strawberry [21]. All these can be developed by lipase induced reactions based on their exclusive specificity, higher rate of reaction and stability in adverse conditions. Transesterification of tributyrin (oil) and hexanol (alcohol) by *Rhizomucor miehei* lipase (Lipozyme IM-77) led to the development of superb fragrance and flavour. In the beverages industry short chain fatty acids are esterified with geraniol and citronellol to develop the excellent flavor [22].

3.4 Lipases in Cocoa butter

Cocoa butter is a fat, contains stearic and palmitic acids and has a melting point of around 37 ºC. Cocoa butter is used to manufacture chocolates. When cocoa butter melts in the mouth during the chewing of chocolates, then it gives a desirable cooling sense. Lipase based on their hydrolysis and esterfication reactions are used to convert the less desirable fats to more value fats in cocoa butter [23], [24]. Immobilized lipase from *Rhizomucor miehei* is used for transesterification reaction, which replaces the low cost palmitic acid found in palm oil with high value stearic acid.

3.5 Lipases in Pulp and paper industry

Enormous amount of lignocellulosic biomass is processed each year by the pulp and paper industry. Historically, enzymes are used in the paper industry for limited uses for eg. modifications of raw starch. The enzymatic pitch control technique using lipases is a routine practice since 1990 for large scale manufacturing of paper. 'Pitch' is the hydrophobic components of timber (mostly waxes and triglycerides), which causes harsh troubles in paper manufacturing process. Lipases are utilized to eliminate the pitch from the pulp produced for paper manufacturing. A pitch control method has been developed by Nippon Paper Industry, Japan, where they utilized lipase of the fungus, *Candida rugosa* for hydrolysis of the timber triglycerides up to 90% [8].

3.6 Lipases in oleochemical industry

Lipases are capable of catalyzing hydrolysis, esterification and transesterification reactions depending upon water content of the reaction mixture [14]. There are huge differences between the chemical and lipase-catalyzed reactions. The lipase-catalyzed reactions can be carried on in weaker conditions than the chemical reactions. This is beneficial because unwanted side reactions such as heat degradation of the substrates can be prevented [2], [3]. The use of lipases in the oleochemical industry reduces thermal deprivation during glycerolysis, hydrolysis, alcoholysis and acidolysis [8]. Oil spills in processing factories, refinery and shore sand could be handled by the use of lipases [25].

3.7 Resolution of racemic acids and alcohols

The stereospecificity as an exclusive property of lipases is broadly used in recognition of racemic organic acid mixtures in immiscible two-phase systems via transesterification and esterification reactions [16]. Lipases are being utilized in resolving racemic mixtures mainly in enantiomers for eg. Anti inflammatory drugs (non steroidal) that are pharmacologically active chiefly in the (S)-enantiomeric form. Therefore, pure (S)-ibuprofen, which is the active form is formulated by hydrolysis and esterification reactions of lipases [26], [27].

3.8 Lipases in tea processing

Lipases are utilized in black tea manufacturing industry. During the process of black tea manufacturing, lipase induces hydrolytic cleavage of membrane lipids and commences the synthesis of volatile products with unique flavour features, emphasizing the significance of lipid in the flavour development. Lipase secreted by *R. miehei* increased the amount of polyunsaturated fatty acid (PUFA) observed by decrease in total lipid content [28].

3.9 Lipases as biosensors

An imperative use of microbial lipases is estimation of lipids in the analytical sample of patients in order to diagnose the cardiovascular complains. The basic concept of quantitative estimation is that lipase catalyzes hydrolysis of triacylglycerol present in the analytical sample into glycerol and free fatty acids. Then the amount of released glycerol or free fatty acids is determined by enzymatic and chemical method. In this way the level of lipids in the patient sample can be determined. *C. rugosa* lipase biosensor system has been developed. Lipase of *C. rugosa* is non specific and allows rapid liberation of glycerol and fatty acids from lipid due to its high specific activity [29].

3.10 Lipases as diagnostic tools

They can be used for diagnosis of diseases because their occurrence or rising levels can specify some infection or disease. Lipases produced by pathogenic bacteria for eg. *Propionibacterium acnes*, *Corynebacterium acnes* [30] and *Staphylococcus aureus* [31] have been reported to have the effect on skin rash in acne (pimples) patients. Medical conditions such as pancreatic injury and acute pancreatitis can be diagnosed by detecting the amount of lipases in the blood serum sample [32]. Level of triglycerides and cholesterol can also be regulated by fungal lipases [33]. Lipases are utilized for the estimation of blood lipid. The basic concept is that lipase catalyzes cleavage of triacylglycerol into glycerol and free fatty acids which are then determined by enzyme conjugated colorimetric reaction. The quantity of free fatty acid reflects the level of triacylglycerol in the analyzed sample [8].

3.11 Lipases in bioconversion in aqueous media

Ester hydrolysis is usually performed with lipase in two-phase aqueous medium. Hydrolysis of *p*NPP in n-heptane using a lipase purified from *P. cepacia* has been reported [34]. Lipase trapped in a hydrophobic sol-gel matrix has been utilized for various transformation reactions [16].

3.12 Lipases in cosmetics and perfumery

Lipases have prospective applications in cosmetics and perfume industry since they exhibit activities in surfactants and in fragrance production [35]. Retinoids (Vitamin-A and derivatives) are of immense commercial potential in pharmaceuticals and cosmetics for eg. skin care products. Immobilized lipase is utilized for the preparation of retinol derivatives (water soluble) [36].

3.13 Biodegradation of plastic

de Castro and Anderson [37] depicted that a technique was invented using lipases by Fermentation

Research Institute Tsukuba, Japan for complete destruction of plastics. This technique was based on the capability of lipases to degrade polycaprolactone (an aliphatic polyester), which can be incorporated into plastics to increase their degradation rate.

3.14 Lipases in medical sector

Lovastatin is a drug used to lower the cholesterol level in serum. This drug is synthesized using catalytic action of lipase from *C. rugosa*. Lipase of *S. marcescens* utilized for asymmetric hydrolysis of 3-phenylglycidic acid ester, is an intermediate in the production of diltiazem hydrochloride, a broadly utilized drug for coronary vasodilation [8], [38].

3.15 Lipases in biodiesel production

The production of biodiesel can be carried out by enzymatic or chemical transesterification of oils/fats. Enzymatic process is preferred over chemical process due to high catalytic activity, stability, cost effectiveness and eco-friendly nature of lipases [39], [40].

3.16 Lipases in organic synthesis

The natural lipases have exceptional regioselectivity and sterioselectivity therefore are exploited for the transformations of enantiomeric compounds [16].

4. CONCLUSIONS

Lipases occur broadly in nature, but only microbial lipases are commercially important. Lipases have different applications such as flavor modification in food industry, ester synthesis in fine chemical industry, fat hydrolysis in detergent industry, biodiesel production, fat modification etc. In last few years attention of industries has been shifted from amylase and proteases to lipases because of their capability to catalyze not only hydrolytic reactions but also esterification and transesterification reactions. Among hydrolytic enzymes, lipases are the enzymes of researcher's choice.

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