International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **1(8),** 88-92, December **(2012)** *Int. Res. J. Biological Sci.*

Review Paper

Microbial Lipases: Industrial Applications and Properties (A Review)

Verma N.[1*], Thakur S.[1] and Bhatt A.K.[2]
[1]Department of Biotechnology, Manav Bharti University, Solan, HP – 173229, INDIA
[2]Department of Biotechnology, Himachal Pradesh University, Shimla, HP – 171005, INDIA

Available online at: www.isca.in
Received 25th September 2012, revised 1st October 2012, accepted 4th October 2012

Abstract

Enzymes are considered nature's catalysts. Lipases are versatile enzymes that are used widely. In fact, over the last few years, there has been a progressive increase in the number of publications related to properties and industrial applications of lipase-catalyzed reactions. So by keeping in view the immense applications of lipase enzyme the present review is focused on properties such as pH and temperature kinetics, stability in organic solvents, effect of metal ions, lipase inhibitors, non-specific reversible inhibitors and various industrial applications including fat and oil processing, food industry, detergents, pulp and paper industry, oleochemical industry, environment management, tea processing, biosensors, diagnostic tools, cosmetics and perfumery and medical applications of lipase.

Keywords: Lipases, organic solvents, inhibitors, oleochemical industry, environment management, biosensors.

Introduction

Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the multibillion dollar underexploited lipid technology bio-industry and have been used in *in situ* lipid metabolism and *ex situ* multifaceted industrial applications[1]. Lipases are triaclyglycerol acylhydrolases (EC 3.1.1.3) that catalyze the hydrolysis of triaclyglycerol to glycerol and fatty acids. They often express other activities such as phospholipase, isophospholipase, cholesterol esterase, cutinase, amidase and other esterase type of activities[2]. Microbial lipases have gained special industrial attention due to their ability towards extremes of temperature, pH, and organic solvents, and chemo-, region-, and enantio-selectivity. Lipases are ubiquitous in nature and are produced by several plants, animals and microorganisms[3]. Some important lipase-producing bacterial genera are *Bacillus, Pseudomonas and Burkholderia*[4] and fungal genera include[5] *Aspergillus, Penicillium, Rhizopus, Candida*. Different species of yeasts belonging to seven different genera include *Zygosaccharomyces, Pichia, Lachancea, Kluyveromyces, Saccharomyces, Candida,* and *Torulaspora*[6]. Knowledge of the three-dimensional structure of lipase plays an important role in designing and engineering lipases for specific purposes. More than 12 lipases from various sources have been crystallized and extensive information on lipase engineering has been documented. All lipases are members of the α/β hydrolase fold super-family. Also, lipases share a conserved active site signature, the Gly-Xaa-Ser-Xaa-Gly motif[7]. Although, considerable progress has been made over the recent years towards the developing cost-effective systems for lipases but the high cost of production of this enzyme remains the major challenge associated with large-scale industrial applications. Given the heterogeneity of natural environments and the enormous potential of microorganisms to provide novel pharmaceuticals, fine chemicals and new technologies, the biotechnology industry has a vast, largely untapped resource for the discovery of new chemicals and novel processes[8].

Properties of Lipases

The number of available lipases has increased since the 1980s and used as industrial biocatalysts because of their properties like bio-degradability, high specificity and high catalytic efficiency. Some unique properties of lipase such as their specificity, temperature, pH dependency, activity in organic solvents and nontoxic nature leads to their major contribution in the food processing industries. Ethyl, isobutyl, amyl and isoamyl acetates are widely used flavor esters[9]. Lipases from different sources have investigated for their hydrolytic as well as synthetic activity. The most desired characteristics of the lipase are its ability to utilize all mono-, di-, and tri-glycerides as well as the free fatty acids in transesterification, low product inhibition, high activity/yield in non-aqueous media, low reaction time, resistance to altered temperature, pH, alcohol and reusability of immobilized enzyme[10]. Additionally, lipases can carry out reactions under mild conditions of pH and temperature and this reduces energy needs to direct reactions at unusual temperatures and pressures. As a result, unstable reactants and products are protected from destruction. Other reasons for biotechnological potential of microbial lipases are their stability in organic solvents and being active without the aid of cofactors[11].

pH and Temperature Kinetics: Bacterial lipases have a neutral or alkaline optimum pH with the exception of lipase from *P. fluorescens* SIK W1 that had an acidic optimum pH 4.8. However, lipases from *Bacillus stearothermophilus* SB-1, *B. atrophaeus* SB-2 and *B. licheniformis* SB-3 are active over a broad[12] pH range 3-12. Bacterial lipases generally have temperature optima in the range[13] 30-60°C. For *B. licheniformis* MTCC-10498 maximum lipase production was observed at pH 7.5 (~0.4 U/ml)[14]. However, bacterial lipases with optima in both lower and higher ranges have been reported. Thermal stability data are available only for species of *Bacillus*, *Chromobacterium*,*Pseudomonas* and *Staphylococcu s*. The thermostability of the enzyme from *Bacillus* sp. was enhanced by the addition of stabilizers such as ethylene glycol, sorbitol, glycerol, with the enzyme retaining activity at even after 150 min of incubation at 70°C[15].

Stability in Organic Solvents: Stability in organic solvents is desirable in synthesis reactions. From the available literature, it can be inferred that lipases are generally stable in organic solvents, with few exceptions of stimulation or inhibition. Ethanol and methanol enhanced the lipase activity of *B. thermocatenulatus* and AG-8 lipase[16].

Effect of Metal Ions: Metal ions can either stimulate or inhibit microbial enzyme production. Metal cations, particularly Ca^{2+}, play important roles in the structure and function of enzymes, and some of the lipases are strictly calcium dependent[17]. Ca2+ ions activated the enzyme, while $Zn2^{+}$, $Fe2^{+}$, $Fe3^{+}$ strongly inhibited its activity. Salt ions like Ca^{2+}, Cd^{2+}, and Fe^{2+} enhanced the activity of immobilized biocatalyst while a few ions like Co^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Al^{3+}, and Na^{+} had mild inhibitory effect[18].

Lipase Inhibitors: Lipase inhibitors have been used in the study of structural and mechanistic properties of lipases. Further, the search for lipase inhibitors is also of pharmacological interest. Lipase inhibitors are used for designing drugs for the treatment of obesity and the problem of acne. Following is an account of general inhibitors. Broadly, inhibitors of enzymes are classified as reversible or irreversible. The reversible inhibitors can be further classified as non-specific and specific reversible inhibitors.

Non-Specific Reversible Inhibitors: Compounds that do not act directly at the active site but inhibit lipase activity by changing the conformation of lipase or interfacial properties are defined as non-specific inhibitors. Surfactants, bile salts[4] and proteins belong to this group of inhibitors. However, surfactants and bile salts activate the enzyme in some cases.

Applications of Lipases

Lipases form an integral part of the industries ranging from food, dairy, pharmaceuticals, agrochemical and detergents to oleo-chemicals, tea industries, cosmetics, leather and in several bioremediation processes[19]. Because of the vast applications, newer microbes are to be screened for production of lipases having desirable properties. The understanding of structure-function relationships will enable researchers to tailor new lipases for biotechnological applications[20].

Lipases in Fat and Oil Processing: Fats and oil modification is one of the prime areas in food processing industry that demand novel economic and green technologies[21]. Fats and oils are important constituents of foods. Lipases allow us to modify the properties of lipids by altering the location of fatty acid chains in the glyceride and replacing one or more of these with new ones. In this way, a relatively inexpensive and less desirable lipid can be modified to a higher value fat. The removal of phospholipids in vegetable oils (de-gumming) using highly selective microbial phospholipases is also a recently developed environmental friendly process[22]. There are many studies on the hydrolysis of fats and oil by lipases used either in the pure form, in the immobilized form or in the cell bound form[23].

Table-1
Industrial Applications[19] of Lipases

Industry	Action	Product of Application
Dairy food	Hydrolysis of milk, fat, cheese ripening, modification of butter fat	Development of flavoring agent in milk cheese and butter
Bakery food	Flavor improvement	Shelf life prolongation
Beverages	Improved aroma	Alcoholic beverages e.g., sake wine
Food dressing	Quality improvement	Mayonnaise dressing and whippings.
Health food	Transesterification	Health foods
Meat and fish	Flavor development	Meat and fish product fat removal
Laundry	Reducing biodegradable strains	Cleaning cloths
Cosmetics	Esterification	Skin and sun-tan creams, bath oil etc
Surfactants	Replaces phosphoilpases in the production of lysophospholipids	Polyglycerol and carbohydrates fatty acid esters used as industrial detergents and as emulsifiers in food formulation such as sauces and ice creams.
Agrochemicals	Esterification	Herbicides such as phenoxypropionate
Pharmaceutical	Hydrolysis of expolyester alcohols	Produce various intermediates used in manufacture of medicine.
Fuel industries	Transesterification	Biodiesel production
Pollution control	Hydrolysis and transesterification of oils and grease	To remove hard stains, and hydrolyze oil and greases.

Lipases in Food Industry: In the field of biotechnology there are many industrial applications that result in biotech products that we use every-day at home. Some of these are food science applications that utilize enzymes to produce or make improvements in the quality of different foods. Lipases have immense application in food industry such as in cheese ripening, flavor development and EMC technology[24]. Lipases are used *ex situ* to produce flavors, and to modify the structure by inter- or transesterification, in order to obtain products of increased nutritional value, or suitable for parental feeding[25]. Lipases have also been used for addition in food to modify flavor by synthesis of esters of short chain fatty acids and a1cohols, which are known flavor and fragrance compounds[26].

Lipases in Detergents: The use of enzymes in detergent formulations is now common in developed countries, with over half of all detergents presently available containing enzymes. Laundry detergents are becoming more and more popular because of their increasing use in washing machine, where it impart softness, resiliency to fabrics, antistaticness, dispersible in water and mild to eyes and skins. There are many different brands or types of laundry detergents, and mostly they claim some special qualities[27]. China's demand for detergent has grown in the past decade and both production and demand will continue to grow in the next five years. There has been a tremendous increase in the significance of the biotechnological applications of lipases since the last two decades where they display amazing versatility in catalytic behavior. The latest trend in detergent industry is towards lower wash temperatures which not only save energy, but also help to maintain the texture and quality of fabrics[28]. Detergent industries are the primary consumers of enzymes, in terms of both volume and value. The use of enzymes in detergents formulations enhances the detergents ability to remove tough stains and making the detergent environmentally safe. Nowadays, many laundry-detergent products contain cocktails of enzymes including proteases, amylases, cellulases and lipases[29].

Lipases in Pulp and Paper Industry: The pulp and paper industry processes huge quantities of lignocellulosic biomass every year. The technology for pulp manufacture is highly diverse, and numerous opportunities exist for the application of microbial enzymes. Historically, enzymes have found some uses in the paper industry, but these have been mainly confined to areas such as modifications of raw starch. The enzymatic pitch control method using lipases have been in use in a large-scale paper-making process as a routine operation since early 1990s[30]. 'Pitch' or the hydrophobic components of wood (mainly triglycerides and waxes), causes severe problems in pulp and paper manufacture. Lipases are used to remove the pitch from the pulp produced for paper making[11]. Nippon Paper Industries, Japan, have developed a pitch control method that uses the *Candida rugosa* fungal lipase to hydrolyze up to 90% of the wood triglycerides.

Lipases in Oleochemical Industry: The current trend in the oleochemical industry involves the use of immobilized lipases to initiate various reactions (hydrolysis, alcoholysis, and glycerolysis) using mixed substrates. Thus, the use of immobilized enzyme ensures high productivity as well as continuous running of the processes. This offers a greatest hope for successful fat splitting/modification without substantial investment in expensive equipment as well as in expenditure of large amounts of thermal energy[24]. The scope for the application of lipases in the oleochemical industry is enormous as it saves energy and minimizes thermal degradation during hydrolysis, glycerolysis, and alcoholysis[23].

Lipases in Environmental Management: Employment of lipases in bioremediation processes is a new aspect in lipase biotechnology. The wastes of lipid- processing factories and restaurants can be cleaned by the help of lipases from different origins. In this sector, lipases could be used by either *ex situ* or *in situ*[31]. Due to the rapid development observed in industries, environmental pollution became more and more critical. Lipase-producing strains played a key role in the enzymological remediation of polluted soils[32]. Cold-adapted lipases have great potential in the field of wastewater treatment, bioremediation in fat contaminated cold environment and active compounds synthesis in cold condition[33] while in temperate regions, large seasonal variations in temperature reduce the efficiency of microorganisms in degrading pollutants such as oil and lipids. The enzymes active at low and moderate temperature may also be ideal for bioremediation process[34].

Lipases in Tea Processing: The quality of black tea is dependent largely on the dehydration, mechanical breaking, and enzymatic fermentation to which tea shoots are subjected. During manufacture of black tea, enzymatic breakdown of membrane lipids initiate the formation of volatile products with characteristic flavor properties, emphasize the importance of lipid in flavor development. Lipase produced by *Rhizomucor miehei* enhanced the level of polyunsaturated fatty acid observed by reduction in total lipid content[35].

Lipases as Biosensors: A promising new field is the use of microbial lipase as biosensors. Biosensors can be chemical or electronic in nature. An important analytical use of lipases is determination of lipids for clinical purpose. The basic concept is to utilize a lipase to generate glycerol from triacylglycerol and quantify the released glycerol or alternatively the non-esterified fatty acid by chemical and enzymatic method. This principal enables physicians precisely to diagnose patients with cardiovascular complaints. Non-specific lipase, especially of *C. rugosa* with high specific activity has been selected to allow rapid liberation of glycerol. *C. rugosa* lipase biosensor, which optically conjugates to bio-recognition group in DNA, has been developed as probe[31].

Lipases as Diagnostic Tools: Lipases are also important drug targets or marker enzymes in the medical sector. They can be used as diagnostic tools and their presence or increasing levels can indicate certain infection or disease. Lipases are used in the enzymatic determination of serum triglycerides to generate glycerol which is subsequently determined by enzyme linked colorimetric reactions. The level of lipases in blood serum can be used as a diagnostic tool for detecting conditions such as acute pancreatitis and pancreatic injury[36]. Acute pancreatitis usually occurs as a result of alcohol abuse or bile duct obstruction. Although serum trypsin level ultrasonography, computed tomography and endoscopic retrograde cholangiopancreatography are the most accurate laboratory indicators for pancreatitis but serum amylase and lipase levels are still used to confirm the diagnosis of acute pancreatitis[37]. Diagnosis of chronic pancreatitis and revealing the presence of exocrine pancreatic insufficiency has also been determined by measuring serum amylase, pancreatic isoamylase, lipase, trypsinogen and elastase[38]. Lipase of pathogenic bacteria such as *P. acnes*[39], *Corynebacterium acnes* and *Staphylococcus aureus*[40] has also been found to have the influence on skin rash in acne patients.

Lipases in Cosmetics and perfumery: Lipases have potential application in cosmetics and perfumeries because it shows activities in surfactants and in aroma[41] production. Retinoids (Vitamin A and derivatives) are of great commercial potential in cosmetics and pharmaceuticals such as skin care products. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase[42].

Lipases in Medical Applications: Lipases isolated from the wax moth *(Galleria mellonella)* were found to have a bacteriocidal action on *Mycobacterium tuberculosis* (MBT) H37Rv. This preliminary study may be regarded as part of global unselected screening of biological and other materials for detecting new promising sources of drugs[43]. Lipase from *Candida rugosa* has been used to synthesize Iovastatin, a drug that lower serum cholesterol level. The asymmetric hydrolysis of 3-phenylglycidic acid ester which is a key intermediate in the synthesis of diltiazem hydrochloride, a widely used coronary vasodilator, was carried out with *S. mmcescens* lipase[44].

Conclusion

Although lipases have several interesting immense applications in the food, detergent, pharmaceutical, leather, cosmetic and paper industries, their industrial uses still remain limited by their high production costs, commercialization in small amounts, and low performance of some lipase-mediated processes. The applications of lipases are broadening rapidly and new applications are still to be explored in these industries.

Acknowledgments

The Author is grateful to Dr. A.K. Bhatt, Associate Professor, HP University Shimla; Ms. Sumita Thakur, Assistant Professor, Manav Bharti University, Solan for their valuable support for writing this review.

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