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LIPASES: AN EFFICIENT BIOCATALYST FOR BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

Lipases (3.1.1.3) are ubiquitous, biogenic enzymes which hydrolyses the triacylglycerols at the water-oil interface. Many species of animals, plants, and microbes produces lipases. The organisms able to produce lipases which could tolerate organic solvents, high temperature, is of industrial interest. Apart from its natural aqueous catalysis it also performs synthetic reactions in non-aqueous conditions, which have undergone tremendous development in last decade and have become a part of growing biotechnological applications. Immobilization of lipases on the particulate carriers have also been widely used for the enzyme's efficient use. Vast industrial applications have been established such as detergent formulations, oleochemical, nutrition, textile, agrochemical industry, food technology, pharmaceutical field and fine chemical processing. Except latter three, most commercial applications do not requisite excessive purification or high degree of purity.

Keywords: Lipases, organic solvent tolerance, immobilization, industrial applications

INTRODUCTION

Lipases (3.1.1.3) are ubiquitous, biogenic enzymes which hydrolyses the triacylglycerols at the water-oil interface. Lipases falls in the class of enzyme Hydrolases (3), Acting on ester bonds (3.1), Carboxylic - ester hydrolases (3.1.1), Esterases (3.1.1.1) and Lipases (3.1.1.3). Lipases are serine hydrolases. There is very small dividing line between the Esterase and Lipase which needs to be understood. Esterases are defined as carboxylesterases which hydrolyse the ester bonds present in the fatty acids of short chain length (≤10 C) whereas lipases catalyse the hydrolysis and synthesis of long chain fatty acids (≥10 C) **(Jaeger and Eggert, 2002; Casas- Godoy *et al.*, 2012)**. Unlike other hydrolytic enzymes, the substrates for the lipases are presented in a separate phase viz. water/oil interphase **(Rubin, 1995; Martinelle *et al.*, 1995)**. Amongst multitude of enzymes, it is one of the enzymes which have a unique property to catalyse the hydrolytic reactions in aqueous as well as synthetic reactions in microenvironment or non - aqueous environment. They hydrolyse the tri-, di- or mono-glycerides into fatty acids and glycerol and also catalyse the synthesis reactions like esterification, interesterification and transesterification (Table 1).

Table 1 Reactions catalysed by lipases

1. Hydrolysis
$R_1COOR_2 + H_2O \leftrightarrow R_1COOH + R_2OH$
2. Esterification
$R_1COOH + R_2OH \leftrightarrow R_1COOR_2 + H_2O$
3. Transesterification
(a) Alcoholysis
$R_1COOR_2 + R_3OH \leftrightarrow R_1COOR_3 + R_2OH$
(b) Acidolysis
$R_1COOR_2 + R_3COOH \leftrightarrow R_3COOR_2 + R_1COOH$
(c) Aminolysis
$R_1COOR_2 + R_3NH_2 \leftrightarrow R_1CONHR_3 + R_2OH$
(d) Interesterification
$R_1COOR_2 + R_3COOR_4 \leftrightarrow R_1COOR_4 + R_3COOR_2$

In cellular metabolism the fatty acids released by hydrolytic reaction can further be utilized for the synthesis of triacylglycerol, act as energy sources and also as precursors for synthesis of membrane phospholipids **(Aschauer *et al.*, 2018)**. For the biotechnological application fatty acids and glycerol thus generated as products can be utilized as a carbon source for the production of different enzymes, as a substrate for the production of mono-, di-, tri- acylglycerol or for production of different products like ethanol and formate by glycerol fermentation **(Binhayeeding *et al.*, 2017; Jarvis *et al.*, 1997)**. Lipases acts on broad range of substrate from various esters of fatty acids to hydrolysing different chain lengths of alcohols.

The characteristics of lipases like regio-, stereo-, and enantio- specificity is one of the major factors for considering this enzyme important for industrial applications **(Kapoor and Gupta, 2012; Sarmah *et al.*, 2018)**.

Regiospecificity

Regio-specificity can also be referred to positional specificity. According to **Matori *et al.*, (1991)**, lipases can be divided into three groups depending on its regio- specificity-

(a) α-specific (1,3 position specific): Lipases which hydrolyse the ester bond at the primary hydroxyl group of the triglyceride, either at position 1 or 3 of a glyceride. This hydrolysis would produce either monoglycerides or diglycerides, latter being much preferred and faster **(Riberio *et al.*, 2011; Kapoor and Gupta 2012)**. Examples are listed in the table 2.

(b) Non- specific: These lipases catalyse reaction randomly, on all hydroxyl groups irrespective of the position on the triglyceride **(Harikrishna and Karanth, 2002; Riberio *et al.*, 2011; Kapoor and Gupta, 2012)**. The nonspecific lipase will act on tri-, di-, and mono- acyl glycerides with the same rates. For biodiesel production, the ideal lipase should be one which has non-regiospecific characteristics, as it can hydrolyse all the three forms of the triglyceride **(Vargas *et al.*, 2018)**.

(c) Fatty acid specific: The lipases which favour the hydrolysis of esters at the specific position of the glyceride chain **(Kapoor and Gupta, 2012)**. For example, the specificity of the mold *Geotrichum candidum* lipase has been studied and results showed, that the enzyme was specific for fatty acids containing cis-9 or cis, cis-9, 12 unsaturated triglyceride regardless of the position **(Jensen 1974; Jensen *et al.*, 1983)**.

Stereospecificity

Stereospecificity of the lipase can be defined as the ability of them to distinguish between sn-1 and sn-3 position on the triglyceride. The lipases preferentially hydrolyse one of the isomers of a racemate mixture over other. This novel characteristic of lipase, is studied extensively and the reports shows this nature acts on various substrates such as straight chain secondary alcohols, acetonoids, inositols, carboxylic acids and several esters of ibuprofens **(Meghwanshi and Vashishtha, 2018)**. According to **Sonnet, (1988)**, the stereoselectivity of the same lipases may vary with the change in the structure of the substrate. The high preference for acting on sn-1 position of trioctanoin substrate by lipase from

Pseudomonas sp. and sn-3 by *C. antarctica* shows high stereospecificity, while all other lipases show low- medium sn-1,3 stereospecificty towards trioctanion **(Kapoor and Gupta, 2012)**.

Table 2 List of organisms and regioselectivity of their lipases

Lipase source	Name of organism	Regio-specificity	Application	Reference
Bacterial	*Anoxybacillus flavithermus* HBB 134	1,3 Regiospecific	-	**Bakir and Metin 2016**
	Staphylococcus sp.	2 Regiospecific	-	**Horchani *et al.*, 2010**
	Chromobacterium viscosum	Non Regiospecific	-	**Riberio *et al.*, 2011**
	Pseudomonas sp.	Non Regiospecific	-	**Riberio *et al.*, 2011**
	Pseudomonas sp.	1,3 Regiospecific	Monoglyceride production, esterification of (R,S)-2-octanol	**Gaoa *et al.*, 2000**
Fungal	*Cordyceps militaris*	1,3 Regiospecific	-	**Park *et al.*, 2019**
	Tricosporon sp.	1,3 Regiospecific	Enrichment of omega 3 polyunsaturated fatty acids	**Cao *et al.*, 2019**
	Rhizopus sp.	1,3 Regiospecific	Interesterification of Amazonian buriti oil and murumuru fat	**Sperenza *et al.*, 2016**
	Rhizomucor endophyticus	1,3 Regiospecific	Biodiesel	**Yan *et al.*, 2016**
	Geotrichum candidum NRRL Y-552	Non-Regiospecific	-	**Laguerre *et al.*, 2017**
	Penicillium simplicissimum, Candida rugosa	2 Regiospecific	-	**Harikrishna and Karanth 2002**
	Geotrichum candidum	Fatty acid specific	-	**Jensen, 1974**
	Candida sp. 99-125	1,3 Regiospecific	Production of 1,3 Diolein	**Bi *et al.*, 2019**

Enantioselectivity

In an enzymatic reaction, the enzyme model always describes the mechanism of enantioselectivity. These models, precisely referred to as rules, only predicts which enantiomer reacts faster, but not the degree of enantioselectivity **(Ghanem and Aboul-Enein, 2005)**. The Prelog's rule **(Prelog, 1964)** was the earliest which predicted the enantioselectivity of the ketones by alcohol dehydrogenases of yeast, on the basis of size of the two substituents on the carbonyl group. Other models are based on pockets, which indicates the size and shape of the molecules present in the active site. **Kazaluskas *et al.*, (1991)** using lipases from *Pseudomonas cepacia* and *Candida rugosa*, put forward a rule to predict the chiral recognition by lipases.

The enantioselectivity by various lipases of different species have been reported. *Pseudomonas fluorescens* and *Bulkholderia cepacia* RQ3 lipase shows the enantioselectivty towards cosmetics and pharmaceutical industry important compound 1-phenylethanol **(Dwivedee *et al.*, 2017; Xie *et al.*, 2016)**.

Structure of lipases

Lipases are serine hydrolases i.e. they contain a serine residue at their active catalytic site. Majority of the Lipases/Esterases holds a small consensus region which is thought to be active site. This region contains G-X-S-X-G sequence which encloses the serine residue, not specifically the same for all enzymes. This presence of the serine residue can be confirmed by chemical modification and site- directed mutagenesis experiments (**Hilton and Buckley, 1991; Davis *et al.*, 1990)**. According to **Murzin's (1995)** SCOP database, the protein structure has been classified into five classes according to the fold types – (a) all α helices, (b) all β sheets, (c) α/β mostly parallel β strands and (d) α/β mostly anti-parallel β strands. This classification also used in Protein Data Base (PDB) for classifying proteins according to their three-dimensional structures. The lipases are categorized in the class α/β mostly with parallel β strands **(Schrag *et al.*, 1997)**. Invention of X-ray crystallography has been proven beneficial for the elucidation of three-dimensional structure of the enzymes. The first three- dimensional structure of two lipases, *Mucor miehei* lipase and human pancreatic lipase, were simultaneously reported in a year **(Brady *et al.*, 1990; Winkler *et al.*, 1990)**. The catalytic triad of serine proteases Ser-His-Asp is well known and similarly the lipases are chemically analogous to, but structurally different from serine proteases. The *Geotrichum candidum* lipase showed Ser-His-Glu catalytic triad, where aspartate is replaced by the glutamic acid **(Schrag *et al.*, 1991)**. Unlike other serine proteases, the sequence of the catalytic triad for lipase should be invariably Ser-Asp/Glu-His **(Schrag *et al.*, 1997)**. As such there is no difference in the structure of lipases obtained from different sources, except the difference in the sequence located near the active sites. A study carried by **Aschauer *et al.*, (2018)** reports the crystal structure of monoacylglycerol lipases from *Mycobacterium tuberculosis*, which shows similarity with human monoacylglycerols. This bacterium was not inhibited by the profound human lipase inhibitor. Also, human and *M. tuberculosis* lipases were marked different by observing the active site through docking. Docking revealed differences in binding pocket which impaired the inhibition.

Different databases are present for the classification and identification of new sequences of lipases and esterases. Lipase Engineering Database (LED) provides the multisequence alignments of all homologous families and superfamilies. The LED integrates the information on sequence - structure - function of lipases/esterases with different proteins of its superfamilies **(Pleiss *et al.*, 2000; Fischer and Pleiss, 2003)**. **Widmann *et al.*, 2010** have done a detailed case study on *Candida antarctica* Lipase A using the LED Database, where they compared the *C. antarctica* Lipase A structure with other superfamilies and found similar structure from the deacetylase family. The ESTHER (Esterases, α/β hydrolase enzymes and relatives) Database aids in comparison and alignment of unknown sequence, structure- function relationship analysis via mutation and structural data retrieval **(Cousin *et al.*, 1996)**. It is expanding new fields for analysis such as generating new families, visualization tools like the family tree and overall table **(Lenfant *et al.*, 2012)**. LIPABASE Database is a repository which is ideal for 'true' lipases from different species. It gives the information about the general, physicochemical, taxonomic and molecular data regarding the 'true' lipases **(Messaoudi *et al.*, 2011)**.

Lipase sources

This industrially important, potent enzyme could be procured through various sources. The sources may include animal, plants and microorganisms.

Plant lipases

For the transesterification of edible oils, specifically when ethanol is used, plant lipases are more advantageous, as ethanol can be produced through fermentation utilizing the plant biomass. Even though, very less focus is given on plant lipases in comparison with the microbial lipases. The majority of the lipases extensively used in biotechnologies are of bacterial and fungal origin **(Nanssou Kouteu *et al.*, 2016; Villeneuve, 2003)**. Oleaginous plants, such as corn, castor bean, sunflower, due to presence of high triacylglycerol content might be the source of lipases. The castor bean seeds and sunflower seeds as the lipase source have been reported by **Tavares *et al.*, (2018)** and **Sagiroglu and Arabaci, (2005)** respectively. The statistical methods were employed to evaluate the effects of physical and media parameters on the hydrolysis yield and the kinetic studies were employed on the castor bean seeds lipase whereas purification and kinetic studies have been studied from sunflower seed lipase. *Jatropha curcus* lipase was able to degrade the phorbol esters, a known toxic agent having potential to cause tumour **(Wardhani *et al.*, 2016)**. Cereals and latex of the fruits are also the major lipase producers. Cereal grains contain the starch or lipids majorly as the nutrient reserve for the energy and utilize it during germination. Rice, wheat, corn are considered to be good lipase producers, showing the presence of lipase activity in different parts of its plant. Wheat germ lipase was initially reported by Singer and Hofstee in 1948 **(Barros *et al.*, 2010)**. The presence of lipase in the rice is mainly in the bran. **Bhardwaj *et al.*, (2001)** identified the first thermostable lipase in rice bran and was found to be glycoprotein. Kinetic parameters and characterization of lipase was studied using purified enzyme and was found to be sn-2 regiospecific. The *Carica papaya* lipase was studied from the latex of the fruit, and was first reported in the 1935 **(Rivera *et al.*, 2012)**. This lipase is on focus and is considered important due to its low cost. A new method was developed by **Nhat and Ha, (2019)** which showed easy isolation of lipase from papaya latex with the help of sodium lauroyl sarcosinate.

Animal lipases

The fish and mammals collectively contribute the lipases from the animal sources. Various organs of fish such as head, frame, the skin, scales and viscera are potentially rich in lipases. The digestive organs which are considered as major by-products of fish industry are also the main sources of lipases and proteases. The fish lipases complement the characteristics of mammalian and microbial lipases, due to its evolutionary pathways (**Kurtovic *et al.*, 2009; Sae-Leaw and Benjakul, 2018; Mardina *et al.*, 2018)**. The microbial lipase expressing system differs from those in mammals in a way such as predominant role of Carboxyl Ester Lipase (CEL)-like enzyme in fat digestion compared to Pancreatic Lipase (PL)-like enzyme **(Kurtovic *et al.*, 2009)**. The lipases from the liver of the seabass was purified and characterized by **Sae-Leaw and Benjakul, (2018)**. The enzyme had higher efficacy of defatting the fish skin as compared to the isopropanol treatment. Apart from fish other marine animals, which are not much studied includes the crustaceans and cephalopods. Earlier, mammals such as pig, rat, cow and sheep were studied to understand the lipid digestion. The studies on porcine pancreatic lipases by **Bier, (1955)**, **Plummer and Sarda, (1973)**, rat pancreatic lipase by **Gidez, (1968)** and bovine pancreatic lipase by **Julien *et al.*, (1972)**, are some of the early researches on the mammalian lipases. The porcine lipase have been applied for the synthesis of aromatic esters such as ethyl oleate, ethyl valerate, butyl acetate **(Ozyilmaz and Gezer, 2010; Hazarika *et al.*, 2002)**. The calcium carbonate immobilized porcine lipase has been used to biodegrade the mycotoxin patulin in apple juice **(Tang *et al.*, 2018)**. The human pancreatic lipase (HPL), whose structure was first predicted with the help of the X-Ray crystallography, has a key role in dietary fat absorption by generating free fatty acids and mono-glycerides from the triglycerides **(Winkler *et al.*, 1990)**. Recent development in HPL is towards developing recombinant HPL, as a supplement for treating the patients with lipase deficiency, for elucidating the properties to control the *in vitro* lipase activity **(Kawaguchi *et al.*, 2018)**.

Microbial lipases

Microbial lipases include the fungal, yeast and bacterial lipases. The reasons why the microbial lipases are biotechnological important are as they (1) are stable at high temperatures, (2) have broad substrate selectivity, (3) have ease of genetic manipulation, (4) are stable in organic solvents, (5) do not require co-factors, (6) abundant and easy growth of microorganisms, and (7) possibility of high yield **(Sharma *et al.*, 2001)**. The table 3 indicates the potent microorganisms producing lipases **(Sharma *et al.*, 2001; Nagarajan 2012)**.

Table 3 List of microorganisms which are potent lipase producers

Source	Microorganism	Source	Microorganism
Fungal	*Rhizopus arrhizus*	**Yeast**	*Candida rugosa*
	Rhizopus chinensis		*Candida cylindracea*
	Aspergillus sp.		*Candida* sp.
	Rhizopus nodosus		*Aureobasidium pullulans*
	Penicillium citrinum		*Saccharomyces cerevisiae*
	Penicillium cyclopium		*Williopsis californica*
	Penicillium wortmanii	**Bacterial**	*Acinetobacter radioresistens*
	Penicillium verrucosum		*Pseudomonas* sp.
	Geotrichum sp.		*Pseudomonas aeruginosa*
	Geotrichum candidum		*Staphylococcus aureus*
	Aspergillus carneus		*Bacillus acidocaldarius*
	Rhizopus sp.		*Bacillus stearothermophilus*
	Aspergillus niger		*Burkholderia cepacia*
	Rhizopus oryzae		*Burkholderia glumae*
	Ashbya gossypii		*Serratia rubidaea*
Yeast	*Rhodotorula mucilaginosa*		*Bacillus* sp.
	Yarrowia lipolytica		*Bacillus coagulans*
	Candida utilis		*Bacillus subtilis*
	Trichosporon asahii		*Bacillus brevis*

Organic solvents tolerance of lipases

For ages, the enzymatic catalysis is being carried out in the aqueous medium. If the enzyme is restricted only to its natural aqueous environment, then many industrial bioconversions, specifically the production of special fine chemicals and polymers are limited, as most of the industrial compounds are hydrophobic. Water may often lead to unwanted side reactions, would degrade organic reagents and also the thermodynamic equilibria of many processes are unfavourable in aqueous medium **(Klibanov, 2001)**. There are various advantages and disadvantages of using enzymes in organic solvent systems (Table 4) **(Doukyu, 2010)**. Natural organic solvent tolerant enzymes are useful for many industrial applications, as there is no need for any modification for the stability of the enzyme. Many organic solvent tolerant lipases have been screened and subsequent characterization has also been done (Table 5). If the enzyme is not naturally potent for solvent tolerance, it can be engineered to function in organic solvents with the activities and selectivities same as would be there in aqueous phase. With the consideration of advantages of non-aqueous conditions, invariably low catalytic activities have been displayed by the enzymes in these conditions compared to the aqueous **(Schimd, 2001)**. The partition coefficient *P* (log *P*) is frequently used to describe solvents effect on the enzyme activity/ stability of the enzyme. **Laane *et al.*, (1987)** have proposed the rules for optimization of biocatalysis in organic solvents which would allow the optimization of any biological reaction in the medium containing various organic solvents. They interpreted that the log *P* correlation for enzymatic conversions is a general phenomenon observed due to differences in the ability of the organic solvents to disrupt the critical water layer around biocatalysts.

Table 4 Advantages and disadvantages of using organic solvent systems for enzymatic catalysis

Advantages	High solubility of hydrophobic substrates
	Catalysis of various reactions that are not attainable in aqueous media
	Thermodynamic equilibria support synthesis reactions over hydrolysis
	Repression of water-dependent side reactions
	Use of different substrates, regio, stereo and enantio-specificity
	Reusability and recovery of enzyme with and without immobilization
	Change of the partition of substrates/products which aids in separations and improved yields
	Increased thermostability in anhydrous organic solvent system
	Enzymes are potent to be used straightly in a chemical process
	Removal of microbial contamination
Disadvantages	Enzyme inactivation
	More labour and costly preparation of biocatalysts in covalently modified systems
	In the case of heterogeneous system, the problems of mass-transfer
	During the involvement of condensation reactions, water activity needed to control
	Solvent toxicity

Immobilization

The discovery of the immobilization technique was first observed using the invertase enzyme, which used the charcoal and aluminium hydroxide as the support material. Such preliminary techniques allowed very less amount of enzyme loading on them. But with time more advanced techniques have evolved which can also be used in the large-scale production processes **(Homaei *et al.*, 2013)**. The primacy of this technique over the enzymes in solutions includes high stability, economically convenient, reusability, effortless product separation, employment of mixed cultures, optimizing the product yield and continuous process can be carried out **(Abdelmajeed *et al.*, 2012; Homaei *et al.*, 2013)**. Immobilization of enzyme means to localize the enzyme onto support. These supports also called as carriers or matrix, may be inorganic or organic. Inorganic matrixes would include-glass, ceramic, silica gel, zirconia, aluminium oxide, nickel oxide and activated carbon. The organic carriers include carbohydrates- cellulose, chitosan, dextran, starch, alginate and proteins- collagen and albumin **(Hettiarachchy *et al.*, 2018)**. Nanoparticles, which have been greatly utilized in the field of medicine and drug delivery have also been explored for the enzyme immobilisation **(De Jong and Borm 2008; Sarno *et al.*, 2017)**. The nanoparticles present a high surface/volume ratio which allows high loading and can also be modified for the easy attachment of enzyme. The attachment is attained through various approaches such as adsorption, covalent attachment, and encapsulation and also through combinations of these **(Sarno *et al.*, 2017; De souza *et al.*, 2017; Kim *et al.*, 2006)**. Table 6 shows the techniques of lipase immobilization used in various studies.

Industrial applications of lipases

This multifaceted enzyme, is one of the crucial enzymes among the class hydrolases for which there are diverse application approaches. Few, from the multitudinous applications are in the food, pharmaceutical, oil and fats, oleochemicals, leather, biodiesel, paper and pulp, dairy, fine chemicals, cosmetics and perfumes industry **(Yvergnaux, 2017; Guerrand, 2017; Melani *et al.*, 2019; Nagarajan, 2012)**. The uses and recent research of lipases in some industries are discussed in detail.

Table 5 Organic solvent tolerant lipases and their applications

Organism	Solubility in Organic Solvent		Applications	Reference
	Stimulatory Effect (≥100% relative activity)	Inhibitory Effect (<90% relative activity)		
Acinetobacter sp. EH28	DMSO, n-Hexane, Acetone	Methanol, DMF, Isopropyl alcohol	Synthesis of ethyl caprylate	**Ahemad *et al.*, 2010**
Idiomarina sp. W33	50% (v/v) of n-Hexane	50% (v/v) of DMSO, Methanol, Ethanol, tert-Butanol, Acetone	Biodiesel production	**Li *et al.*,2014**
Pseudomonas aeruginosa AAU2	50 % (v/v) of Iso-Octane, Xylene	50 % (v/v) of n-Heptane, n-Hexane, Toluene, Benzene, Chloroform, Dichloromethane, Methanol	Biodiesel production, Phorbol ester degradation	**Bose and Keharia 2013**
Pseudomonas aeruginosa LX1	25 % (v/v) of n-Hexadecane, Iso-octane, Glycerol	25 % (v/v) of Methanol, Ethanol, tert-Butanol, Acetone, Acetonitrile	Biodiesel production	**Ji *et al.*, 2010**
Pseudomonas stutzeri LC2-8	25 % (v/v) of Isopropanol, Acetone, Methanol, ethanol, DMF, DMSO	25 % (v/v) of Nonane, Iso-octane, n-Octane	Kinetic resolution of (R, S)-1-phenylethanol	**Cao *et al.*, 2012**
Actinomadura sediminis UTMC 2870	20 % (v/v) Methanol, Dimethyl formamide, DMSO, Propanol, Cyclohexanol	20 % (v/v) Ethanol, Acetone, Formaldehyde	omega-3 production	**Imanparast *et al.*, 2018**
Burkholderia cepacia RQ3	25 % (v/v) Hexadecane, Tetradecane, Dodecane, Isopropanol, Ethanol	25 % (v/v) Hexane, DMSO	Chiral resolution of 1-phenylethanol	**Xie *et al.*, 2016**
Bacillus atrophaeus FSHM2	25 % (v/v) Xylene, n-Hexane, Isoamyl alcohol, Isopropanol	25 % (v/v) Methanol, Acetone, Chloroform, DMSO	Synthesis of methyl and ethyl valerate	**Ameri *et al.*, 2017**

Detergent Industry

Owing to the property of catalysing the reaction in the aqueous phase, lipases along with the proteases and amylases are used to increase the efficacy of the detergents. The lipase use could reduce the washing time, without adjusting the temperature and the agitation **(Agobo *et al.*, 2017)**. Lipases which are thermophilic, thermostable, alkaline, water soluble, resistant to the detergent proteases and also could retain its properties in liquid as well as powdered detergents are considered to be used in the detergent industry **(Sarmah *et al.*, 2018; Chauhan *et al.*, 2013; Zarinviarsagh *et al.*, 2017)**. Use of psychrophilic lipase along with biosurfactant was carried out and patented by **De Rose *et al.*, (2018)**, in the detergent formulation. The enzyme was effective in the temperature range from 0°C to 25°C. The *Ochrobactrum intermedium* strain MZV101 isolated by **Zarinviarsagh *et al.*, (2017)**, showed the production of lipase along with the biosurfactant. The enzyme was active even in absence of Ca^{+2} and was found successful in detergent application. In contrast to this the lipase from *Burkholderia cepacia* RGP-10 showed good activity in presence of Ca^{+2} and was compatible with various non-ionic, ionic and laundry detergents **(Rathi *et al.*, 2001)**. A surfactant and detergent stable lipase isolated from the Pacific white shrimp, showed very good compatibility with the liquid and the powdered detergents **(Kuepethkaew *et al.*, 2017)**. Some of the lipases with potential use in the detergent industry can be produced from *Staphylococcus aureus*, *Serratia marcescens*, *Bacillus methylotrophicus* PS3 and *Trichoderma lentiforme* ACCC30425 **(Bacha *et al.*, 2018; Garcia-Silvera *et al.*, 2018; Sharma *et al.*, 2017 and Wang *et al.*, 2018)**.

Table 6 Immobilization of lipases using different support and their application

Sr. No.	Name of organism	Immobilization support	Application	Reference
1	*Pseudomonas stutzeri* and *Alcaligenes* sp.	Hydrophilic-hydrophobic porous silica	Synthesis of sugar ester -lactulose palmitate	**Bernal *et al.*, 2014**
2	*Candida antarctica* and *Rhizomucor miehei*	Hydrophobic chitosan	Hydrolysis of fish oil	**Urrutia *et al.*, 2018**
3	*Pseudomonas fluorescens*	Functionalized multiwalled carbon nanotubes	Kinetic resolution of (RS)-1-phenylethanol to (S)-1-phenylethanol	**Dwivedee *et al.*, 2017**
4	*Candida rugosa*	Magnetic collagen fiber	Synthesis of esters- butyrate esters	**He *et al.*, 2017**
5	*Thermomyces lanuginosus*	Nonporous polystyrene	Hydrolysis of soyabean oil	**Dantas *et al.*, 2019**
6	*Burkholderia ambifaria* YCJ01	Mesoporous TiO_2	Synthesis of ester- cinnamyl acetate	**Gao *et al.*, 2018**
7	*Candida antarctica*	Polyporous magnetic cellulose beads	Biodiesel synthesis	**Zhang *et al.*, 2020**
8	*Candida antarctica*	Hydrophobic virus-like organosilica nanoparticles	Esterification reaction –levulinic acid and n-lauryl alcohol	**Jiang *et al.*, 2019**
9	*Candida rugosa*	Magnetic nanoparticle	Synthesis of pentyl valerate	**Yi *et al.*, 2017**
10	*Candida antarctica* and *Candida rugosa*	Silica nanoparticles	Glycerolysis – olive oil and glycerol	**Singh and Mukhopadhyay 2018**

Biodiesel

As the conventional energy sources such as natural gas, oil and coal which plays a major role in the economic progress, are also getting depleted very rapidly. Apart from this, these natural resources cause a serious environmental issue, which encourages the research for development of new greener and efficient bio-resource **(Ellabban *et al.*, 2014)**. So, Biodiesel can be considered as an alternative. It can be produced from vegetable oils, tallow, non-edible plant oils, animal fats and waste cooking oils. Compared to conventional diesel it emits less air pollutants, greenhouse gases and is non-toxic. However, with all these positive points, biodiesel could not be used extensively as other conventional resources due to its high cost of production. So, researchers should focus on increasing the productivity with the use of low cost of raw materials **(Gebremariam *et al.*, 2018)**. Biodiesel can be produced in two ways by chemical and biological means. The alkali and/or acid catalysts is used by the chemical medium while lipases by biological medium. Presently, there are substantial reports regarding the enzyme mediated biodiesel production, and based on its application form of enzymes, the associated research can be classified into, whole cell catalyst, immobilized lipase and liquid lipase mediated esterification for the biodiesel production (**Du *et al.*, 2008; Chen *et al.*, 2018)**. The hindrance of negative effect of the side product glycerol, during the biodiesel production was overcome by introducing dimethyl carbonate in the methanolysis which reacts with glycerol and releases methanol simultaneously **(Tian *et al.*, 2018)**. **Rana *et al.*, (2018)**, had isolated a lipolytic strain *Bacillus subtilis* strain Q1 KX712301 which catalysed the methanolysis by utilizing a non–edible oils. Other lipolytic strains capable of biodiesel production are *Pseudomonas aeruginosa* BUP2, *Bacillus licheniformis* KM12, *Lasiodiplodia theobromae* and *Pseudomonas cepacia* **(Panichikkal *et al.*, 2018; Malekabadi *et al.*, 2018; Venkatesagowda *et al.*, 2018; Li *et al.*, 2018)**.

Food Industry

One of the necessities of the lipases in this industry is to improve the organoleptic properties of the products. The esters formed by the esterification reaction carried out by the lipases between the short and medium chain carboxylic acids and alcohol produces variety of flavour and aroma components. The Benzyl, anisyl, cinnamyl, cresyl, benzoate, eugenyl and cinnamate esters are considered to be chief aromatic esters **(Agobo *et al.*, 2017; SÁ *et al.*, 2017)**. Isoamyl acetate has the essence of banana and is naturally found in them. The *Bacillus aerius, Candida antartica lipase* B and *Burkholderia cepacia* lipases have been reported to syntheses the isoamyl acetate flavour **(Narwal *et al.*, 2016; Nyari *et al.*, 2018; Padilha *et al.*, 2018)**. *Yarrowia lipolytica* lipase was used for the synthesis of a wide range of flavour esters, in which highest conversion was seen of ethyl octanoate followed by ethyl decanoate and cinnamyl acetate **(de Souza *et al.*, 2019)**. Other sections in food industry where lipases have been used are in egg yolk treatment, edible oil production, in modification of lecithin and in degumming of oils **(Guerrand, 2017; Gerits *et al.*, 2014)**. The lipases also preferentially hydrolyse the ethyl esters of polyunsaturated fatty acids (PUFAs) for the enrichment of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from the fish oils. DHA is an essential polyunsaturated fatty acid, which is used to prevent several human diseases, such as inflammation, cancer, allergy, cardiovascular diseases and renal disorders **(Castejón and Señoráns, 2019)**. *Trichosporon* sp. F1-2, 1,3 regioselective lipase having a good tolerance to different solvents, was applied as a catalyst for enrichment of DHA and EPA in fish oils **(Cao *et al.*, 2019)**.

Pharmaceutical and medical industry

As lipases catalyse wide variety of reactions and have distinct properties like regio-, stereo-, enantio- selectivity, have wide substrate recognition and ability of catalysing reactions in organic solvents, they are of utmost importance in the pharmaceutical and medical industries. The drugs synthesized by lipases are mainly used to cure the diseases like obesity, inflammation, anxiety, pain and cardiovascular **(Melani *et al.*, 2019)**. In this field, the lipases are used to synthesize enantiomerically pure active pharmaceutical products and their intermediates. The applications of lipases in enantioselective synthesis include desymmetrization and kinetic resolution **(Gotor-Fernández *et al.*, 2006; Carvalho *et al.*, 2015)**. An approach of kinetic resolution of racemic 1-phenylethanol has been used as this compound is important in both pharmaceutical and cosmetics industries. *Burkholderia cepacia* RQ3 and *Pseudomonas fluorescens* lipases have been reported to synthesize the 1-phenylethanol from the racemic mixture **(Xie *et al.*, 2016; Han *et al.*, 2016)**. Among majority of enzymes, lipases are frequently used for profen biotransformation. Profens lies under the nonsteroidal anti-inflammatory drugs, popularly practiced for the pain and inflammation caused in an injury. Between the (R) and (S) enantiomer of profens, most of them such as ketoprofen, flurbiprofen, ibuprofen are seen to be (S)-enantiomer, and is pharmacologically more active **(Sikora *et al.*, 2014)**.

CONCLUSION

The lipases have emerged as a versatile enzyme through the years, due to its properties like regio-, stereo- specificity, enantio- selectivity, solvent stability, compatibility with various substrates for the immobilization and exhibits enormous applications in diverse fields like of detergent, food, biodiesel, cosmetics, pharmaceutical and many more industries.

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