LIPASE CLASSIFICATION AND ITS BIOTECHNOLOGICAL APPLICATIONS

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***Abstract*:** Lipases are the water soluble enzymes which have the ability to hydrolyse triacylglycerols to release free fatty acids and glycerol. Lipases constitute a major group of biocatalysts that have immense biotechnology applications. Lipases can be isolated from fungi, yeast, bacteria, plant and animal sources, out of all these, the bacterial lipases are more economical and stable. . Lipases show huge applications in the industries of fat and oil processing, food industry, detergents, pulp/paper industry, environment management, biosensors and cosmetics/perfumery. Microbial lipases are used extensively in food and dairy industry for hydrolysis of milk, fat, cheese ripening, flavour enhancement and lipolysis of butter, fat and cream. Lipases are also used in detergent industry as additive in washing powder, textile industry to increase fabric absorbency, for the synthesis of biodegradable polymers or compounds and different transesterification reaction. In addition this enzyme is used as catalyst for production of different products used in cosmetic industry, in pulp and paper industry, in synthesis of biodiesel, degreasing of leather and pharmaceutical industry.

Keywords: Lipase, hydrolase, transesterification, food industry

1. Introduction

Lipases are classified as hydrolases (glycerol ester hydrolase, E.C. 3.1.1.3) and these enzymes act on ester bonds of several compounds, with acylglycerols being the most suitable substrates,. It catalyze hydrolysis, synthesis, and trans- and inter-esterification reactions (**Jaeger, K.E. et. al. 1994).** Mode of action of lipases as a biocatalyst is shown below (Fig 1):

Fats (or) Oils + Water ──lipase──► Fatty acids + Glycerol

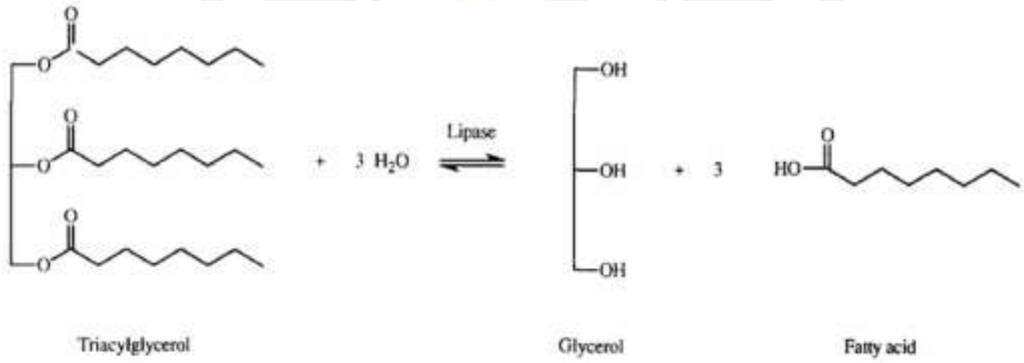

Figure 1: The catalytic action of lipases: A triglyceride can be hydrolysed to form glycerol and fatty acids, or the reverse (synthesis) reaction can combine glycerol and fatty acids to form the triglyceride (Adapted from Jaeger, K.E., et al., 1994).

Lipases are water soluble enzymes which hydrolyze triacylglycerol to release free fatty acid and glycerol, which can be used by the body as energy source. Lipases are more active in insoluble substrates; especially triglycerides made of long-chain fatty acids with over 10 carbon atoms, lipases need a minimum substrate concentration to show high activity levels. Lipases can be isolated form a wide range of sources including Animals, Plants, Insects and Microorganisms (fungi, yeast, and bacteria). The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains. Lipase-producing microorganisms have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairy plants, and soil contaminated with oil and oilseeds among others (Jaeger, K.E., et al., 1994).

Commercially useful lipases are mainly obtained from microorganisms. Fungal Lipase is a specific enzyme that digests fat and is characterized by its ability to hydrolyze fat over a wide range of temperatures and pH. Bacteria produce different classes of lipolytic enzyme, including carboxylesterases, which hydrolyze small ester containing molecules at least partly soluble in water, true lipases, which display maximal activity towards water insoluble long-chain triglycerides, and various types of phospholipids. The fat decomposition for the microorganisms is a source of carbon and it begins through lipase enzyme acting on the fats and is accompanied by the formation of glycerol, fatty acids. Lipases possess the ability to hydrolyze fats into fatty acids and glycerols at the water-lipid interface and can also reverse the reaction in non-aqueous media. (Barros, M., et al 2010).

2. Classification of lipases on the basis of specificity

According to Macrae and Hammond (1985) and Sonnet (1988), lipase can be divided into three main groups as follows based on its specificities:

Substrate specificity: the natural substrates are glycerol esters. The enzyme catalyzes the hydrolysis of triacylglycerols (TAGs), dimonoacylglyerols, monoacylglycerols and phospholipids (phospholipases) (Fig 2.).

Regioselective – subdivided into:

a) Non-specific lipases: catalyse the complete hydrolysis of random triacylglycerols into fatty acids and glycerol via mono- and diacylglycerols as intermediate products.
b) Specific 1.3 lipases: triacylglycerols hydrolysis at the C1 and C3 glycerol bonds, producing fatty acids and chemically unstable products such as 2-monoacylglycerols and 1.2- or 2.3-diacylglycerols.
c) Specific or selective type fatty acid lipases: can be specific for a particular fatty acid/ group of fatty acid. Fatty acid esters located at any triacylglycerol position can be hydrolysed.

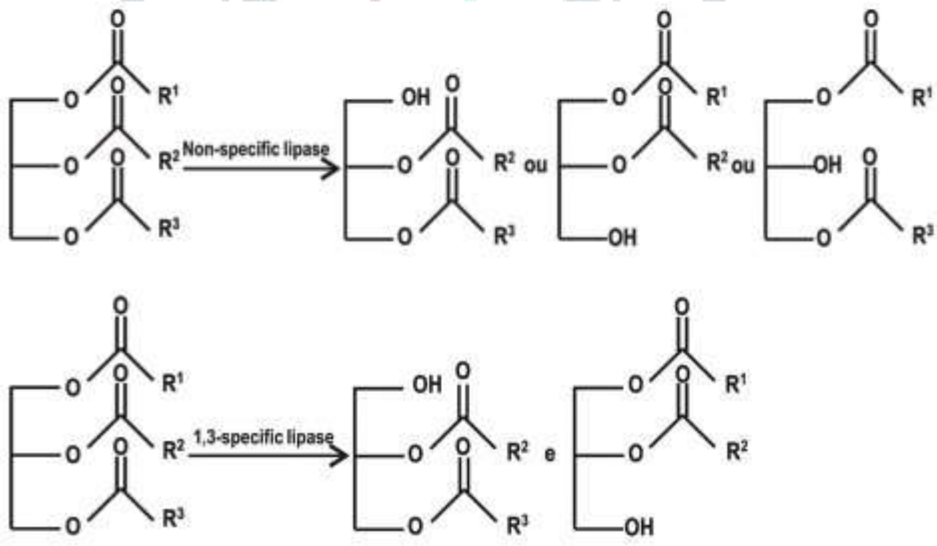

Figure 2: Reaction Catalysed by non-specific and 1.3 specific lipases (Adapted from Barros, M., et al 2010).

Enantioselective: The ability to distinguish enantiomers in a racemic mixture. The enantio specificities of lipases can vary according to the substrate and this variation is due to the chemical nature of the ester.

3. Mechanism of action of lipase

The reaction starts with a nucleophilic attack on the carbon involving the ester bond of the susceptible substrate by hydroxyl group in the serine residue of the active site, forming an acyl-enzyme complex followed by release of alcohol from the lipid. Later, the acyl-enzyme complex is hydrolysed, releasing the lipase regenerated (Fig 3). The enzymatic activity of lipase greatly increase when it contacts the lipid-water interface, a phenomenon called interfacial activation. The active site of the 449-residue present in the enzymes N terminal domain, has a catalytic triad that closely resembles that of the serine protease.

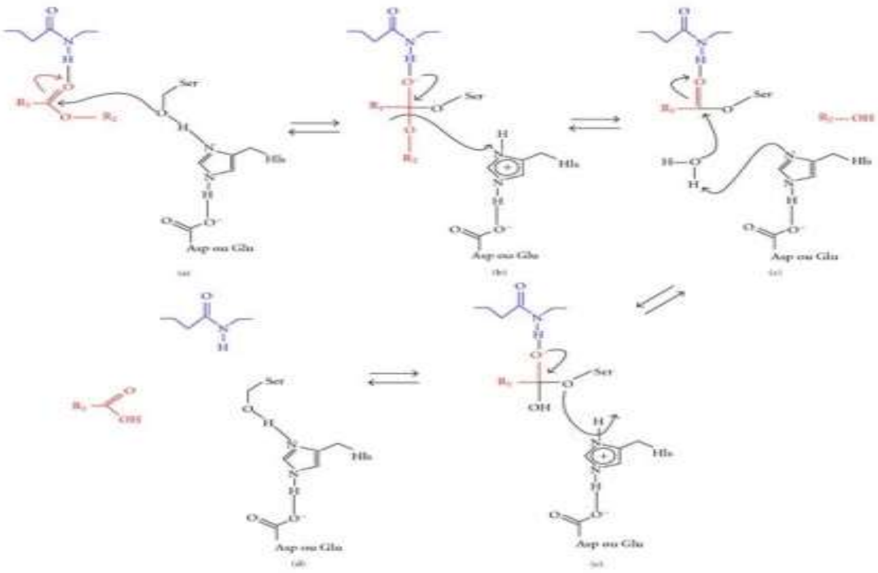

Figure 3: Mechanism of the hydrolysis reaction of ester bonds by lipases (Adapted from Ribeiro, B.D. et al, 2011)
(a) Nucleophilic attack of serine hydroxyl on the carbonyl carbon of susceptible ester bond;
(b) Tetrahedral intermediate;
(c) Acyl-enzyme intermediate and nucleophilic attack by water;
(d) Tetrahedral intermediate;
(e) Free enzyme.

In aqueous solution the lipase's active site is covered by a 26-residue helical lid. However in the presence of the mixed micelles, the lid undergoes a complex structural reorganisation that expose the active site; causes a contacting 10-residue loop, the β5 loop, to change conformation in a way that form the active enzyme's oxyanion hole; and generates a hydrophobic surface about the entrance to the active site (Ribeiro, B.D. et al, 2011).

4. Sources of lipases

Lipases occur widely in nature and can be produced by many microorganisms and higher eukaryotes. In animals, lipases obtained from pig and human pancreas are best known and more investigated than all other lipases. In plants, lipases are present in higher plants seeds, as castor bean and canola (*Brassica napus*). They are also found in several plants' energy reserve tissues. Lipase was first discovered in 1856 in pancreatic juice by Claude Bernard. Animal pancreatic extracts were traditionally used as the source of lipase for commercial applications. The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains. Lipase-producing microorganisms have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairy plants, and soil contaminated with oil and oilseeds among others. Microbial sources are superior to plants and animals for enzyme production and this can be attributed to the ease with which they can be mass cultured and genetically manipulated. Bacterial strains are being constantly screened and improved for lipase production.

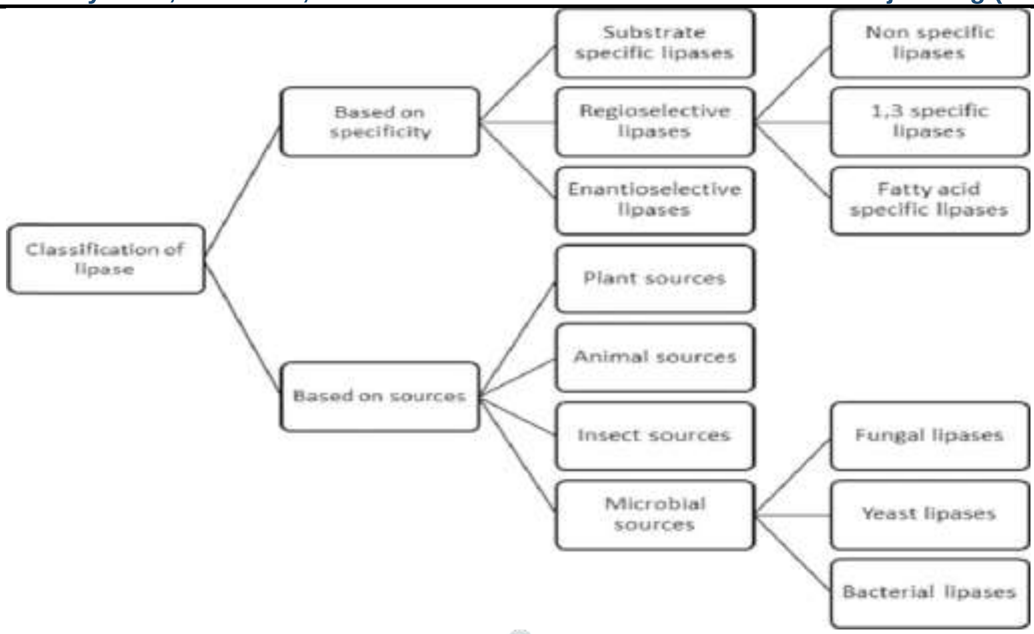

Figure 2: Classification of Lipase on the basis of specificity and sources (Adapted from Arpigny, J.L. and Jaeger, K.E., 1999).

4.1 Animal-based lipases

Animal-based enzymes function exclusively within a limited pH level range, which renders them fairly ineffective in the gut. They become unstable in a low pH level (acidic) environment, resulting in the enzyme being destroyed before it can perform its function. However, for the reasons outlined above, the general consensus is the best sources of enzymes are plant and fungal.

Animal Lipases can obtain from two primary sources: Edible fore stomach of calves, kids, and lambs and Animal Pancreatic tissues.

These preparations may be purified edible tissues preparations or they may be aqueous extract. Most lipases originated from cow and pig pancreatic glands as well as the pregastric juices of calves, lambs, or baby goats.

Human pancreas, Human Gastric cells, Porcine pancreas, Guinea pig pancreas, *Cyprinionmacrostomus*, Chicken adipose cells, Scorpion, Rainbow trout, *Dasyatispastinaca*, Seabass liver are the common sources of Animal and Insect Lipases.

The main triglyceride-lipase (TG-lipase) from the fat body of *Manduca sexta* is a conserved protein among insects and shares major sequence similarity with vertebrate phospholipases (PLs) from the phosphatidic acid preferring-phospholipase A1 (PA-PLA (1)) family.

4.2 Plant–Based Lipases

Lipases appear to have wide distribution in the plant kingdom. High lipase content belong to the families Euphorbiaceae, Ranunculaceae and Papaveraceae. The seeds from grasses that produce the cereal grains are also good sources of the enzyme. The two that have been studied the most are oat lipase and wheat germ lipase. The highest lipase activity of the vegetative portion was relatively low as compared to the generally high values in the seeds.

Fruits and vegetables are commonly consumed in their raw, natural form. Additionally, plant-based digestive enzymes are effective over a broad scope of pH levels. This range is generally believed to be between 3.0 and 9.0, which is highly compatible with the human gastrointestinal environment.

In germinated oilseeds, mobilization of the stored fatty acid is essential to supply energy and carbon for embryonic growth. Lipolytic enzymes catalyse the first step of lipid mobilization, with the possibility of subsequently being controlled during and after the germination period (Barros, M., et al 2010).

4.3 Micro-organisms–Based Lipases

Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources. However, microbial sources are preferred for industrial lipases, due to their short generation time, high product yield, substrate conversion rate, tolerability to the environmental conditions and, easy genetic manipulation (Thakur, S., 2012). Due to habitats' multiplicity, microorganisms usually produce various lipases types (Table 1), with distinct specificity regarding to substrate utilization and also to optimum pH and temperature range.

4.3.1 Fungal–Based Lipases

Fungal Enzymes have numerous uses. They are critical in the production and preparation of many food products, like beer, soy sauce, miso, and baked goods, dairy and processed fruit. One of the most popular and well known culinary fungi is the mushroom. Some mushroom species produce enzymes, including hydrolases, esterase, and phenol oxidases.

Fungi can contain a variety of enzymes, such as protease, amylase, lipase, cellulase and tilactase. Like plant enzymes, fungal enzymes are acid stable and can survive within the pH range of the stomach. They are also suitable for a vegetarian diet, unlike animal-sourced enzymes.

Commercially significant lipase-producing fungi belong to the genera *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp.and *Rhizomucor* sp.

4.3.2 Yeast-Based Lipases

Common lipase producing yeast belong to *Candida* species including *Candida tropicalis, Candida antarctica, Candida cylindracea, Candida curvata, Candida valida.* Other prominent producers are *Pichia bispora, Pichia mexicana, Pichia sivicola, Pichia xylosa, Pichia burtonii, Saccharomycopsis crataegenesis,Trichosporon asteroids* and *Torula sporaglobosa*, (Thakur, S., 2012).

Table 1: Potential Lipase Producing Microorganisms (Adapted from Thakur, S., 2012).

Microorganisms	Source
Acinetobacter radioresistens	Bacterial
Aspergillus carneus	Fungal
Aspergillus niger	Fungal
Aspergillus sp.	Fungal
Aureobasidium pullulans	Yeast
Bacillus coagulans	Bacterial
Bacillus sp.	Bacterial
Bacillus stearothermophilus	Bacterial
Bacillus subtilis	Bacterial
Burkholderia cepacia	Bacterial
Burkholderiamultivorans	Bacterial
Candida cylindracea	Fungal
Candida rugosa	Fungal
Candida utilis	Fungal
Colletotrichum gloesporioides	Fungal
Geotrichumcandidum	Fungal
Geotrichum sp.	Fungal
Penicillium citrinum	Fungal
Penicillium restrictum	Fungal
Penicillium simplicissimum	Fungal
Penicillium verrucosum	Fungal
Pseudomonas aeruginosa	Bacterial
Pseudomonas sp.	Bacterial
Rhizopus arrhizus	Fungal
Rhizopus chinensis	Fungal
Rhizopus homothallicus	Fungal
Rhizopus oryzae	Fungal
Rhizopus sp.	Fungal
Rhodotorulamucilaginosa	Yeast
Rhodotorulamucilaginosa	Yeast
Saccharomyces cerevisiae	Yeast
Serratia rubidaea	Bacterial
Staphylococcus caseolyticus	Bacterial
Williopsis californica	Yeast
Yarrowialipolytica	Yeast

Many microorganisms have been reported in as lipase producers; the genera *Candida, Rhizopus, and Pseudomonas* are considered the main industrial sources of lipases. The yeast *Candida rugosa* is the most employed microorganism for lipase production.

4.3.3 Bacterial lipases

Bacterial Lipases is group of enzyme with one of the major technological interests because of its stability in the presence of organic solvents, no necessity of cofactors for their action and high enantioselectivity (Table 2).

Bacterial lipases were first obtained in 1901 from the strains *Serratia marescens* and ***Pseudomonas aeruginosa*** (Hasan ***et*** *al*., 2006). Production of bacterial lipase particularly from *Pseudomonas* and *Bacillus* sp., for example *P. aerugin* (Madan and Mishra, et al., 2010), *Pseudomonas fluorescens* (Yang *et al*., 2009), *B. pumilus* (Sangeetha et al., 2010a), *B. thermocatenulatus* (Quyen et al., 2003), *B. subtilis* (Ahmed et al., 2010), *B. licheniformis* (Sangeetha et al., 2010a), *B. cereus* (Dutta and Ray, 2009) and *B. halodurans* (Ramchuran et al., 2006) has been well documented. Other genera like *Acinetobacter Staphylococcus* (Talon et al., 1996), *Streptococcus* (Tripathi et al., 2004), *Burkholderia* (Wang et al., 2009), *S. marescens* (Long et al., 2007), *Achromobacter*, *Arthrobacter*, *Alcaligens* and *Chromobacterium* (Riaz et al., 2010) have also been studied.

Lipases belong to the family of serine hydrolases and their activity relies on a catalytic triad comprising of serine, histidine and aspartate and α/β hydrolase fold. Bacterial lipolytic enzymes were classified into 8 families and the largest family was subdivided into 6 sub-families (Arpigny and Jaeger, 1999).

Table 2: Common lipase producing Bacteria (Adapted from Gupta, R. et al 2004)

Thermostable lipase producing microorganisms	Psychrophilic lipase producingmicroorganisms
P. fluorescens	*Aeromonas* sp.
Bacillus coagulans	*Pseudoalteromonas* sp.
P. furiosus	*G. candidum*
Bacillus stearothermophilus	*Psychrobacter* sp.
P. aeruginosa	*C. antarctica*
A. sobria	*Pseudomonas* sp.
Geotrichum sp.	*Rhizopus* sp.
Geotrichum sp.	*Mucor* sp**.**

5. Structure of lipase

Lipase structures have been investigated using X-ray crystallography in both open and closed conformations. Microbial lipases are 20–60 kDa proteins. All the lipases have similar three-dimensional structure which is characterized by α/β-hydrolase folding (a specific sequence of α-helices and β-strands) with most of them containing a helical segment known as the lid which covers the active site while the enzyme is in the closed conformation.

When lipid aggregates are available, the lid get opens immediately and the enzyme activity increases, the condition is regarded as interfacial activation. The lipase core is mostly composed of a central β sheet that is composed of eight different β strands (β1-β8) connected by up to six α helices (A-F). Canonical fold of α/β-hydrolase is present here. The canonical α/β-hydrolase fold consists of a central, mostly parallel β sheet of eight strands with the second strand anti-parallel. The parallel strands range from β3 to β8 is connected by α helices packing either side of the central β sheet. The β sheet possess a left-handed super-helical twist covering the surface of the sheet for about half a cylinder while, the first and last strands crossing each other at an angle of 900. The curvature of the β sheet may differ extensively among different enzymes and also, the spatial positions of topologically equivalent α helices may vary greatly. They differ substantially in length and architecture, in agreement with the large substrate diversity of these enzymes **(Mala, J, et al 2008)**. The active site of the α/β-hydrolase fold enzymes composed of three catalytic residues which are referred as nucleophilic residue (serine, cysteine, or aspartate), a catalytic acid residue (aspartate or glutamate) and a histidine residue.

Serine residue is the nucleophile in lipases, while, the catalytic acid is either an aspartate or a glutamate residue. The nucleophilic Ser residue resides at the C-terminal end of strand β5 in a conserved penta-peptide GXSXG, forming a characteristic β-turn-α motif ('nucleophilic' elbow). The hydrolysis of the substrate is started with a nucleophilic attack by the catalytic-site-Ser oxygen on the carbonyl carbon atom of the ester bond, resulting in the formation of a tetrahedral intermediate stabilized by hydrogen bonding to nitrogen atoms of main chain residues that belong to the so-called 'oxyanion hole'. An alcohol is produced and released from an acyl-lipase complex which is finally hydrolysed with the production of the fatty acid and regeneration of the enzyme **(Donald, V., Donald, V., Voet, Fundamentals of Biochemistry Life at the Molecular Level, 2006)**. The lipase from *Bacillus subtilis* depicted a globular (single) compact domain with dimensions of 35 × 36 × 42 Å. Its fold conformed to the α/β hydrolase, although it lacked the β1, β2 strands of the canonical fold. The active site triad consists of Ser78, Asp134 and His157. Glycosylation is an important feature of eukaryotic lipases, a distinct characteristic of the higher order. Glycosylation is known to contribute to the stability of lipase but does not affect the enzyme activity (Mala, J.G.S. and Takeuchi, S., 2008).

6. Lipase immobilization

Lipases are readily and often used in biotechnological processes. Their activity may be improved by immobilization. Because of this, new studies are conducted which pertain to the activity and application of immobilized lipase enzyme.

There exist many methods of immobilizing lipase, starting from adsorption or precipitation on hydrophobic material, covalent bonding to functional group, and trapping in polymer gels, adsorption on macro-porous, anionic ion-exchange resins, or microencapsulation in lipid membranes. Among the mentioned methods, covalent bonding of lipase is dominating (Sangeetha, R., et al., 2011).

Candida cylindracea and *Candida rugosa* lipase covalently immobilized on silica and aluminium oxide were characterized by higher activity and thermal resistance than pure enzymes or even those immobilized on porous glass. Commonly two bonding factors can be used for covalent immobilization of lipase-glutamate aldehyde and 1, 6-diaminohexane.

7. Industrial Applications of lipases

Lipases are used extensively in food/dairy industry for hydrolysis of milk, fat, cheese ripening, flavour enhancement and lipolysis of butter, fat and cream. Bacterial lipases are also used in detergent industry as additive in washing powder, textile industry to increase fabric absorbency for the synthesis of biodegradable polymers or compounds and different transesterification reaction. In addition this enzyme is employed in the production of various products in cosmetic industry, pulp/paper industry, synthesis of biodiesel, degreasing of leather and pharmaceutical industry **(Sirisha E., et al., 2010).**

7.1 Lipases in fat and oleo-chemical Industry

As lipase hydrolyse lipids to obtain fatty acids and glycerol, which have important industrial applications. Its byproducts for instance, fatty acids are used in soap production **(Hasan, F, et al, 2006).** The lipase catalysed transesterification in organic solvents is trending industrial application wherein by the trans-esterification of less valuable fats can be done to convert them into valuable form, including cocoa butter equivalent, human milk fat substitute (Betapol), polyunsaturated fatty acids (PUFA) rich/low calorie lipids, "designers' fats or structured lipid" and production of biodiesel from vegetable oils.

Mucor miehei and *Candida Antarctica* lipases can be employed for esterification reaction of free fatty acids in the lack of organic solvent or transesterification of fatty acid methyl esters in hexane with isopropylidene glycerols.

A list of reactions catalysed by Lipases derived from bacterial Sources, fungal sources, yeast sources and their industrial applications are mentioned in Table 3, Table 4 and Table 5 respectively.

Table 3: Reactions Catalysed by Lipases Derived from Bacterial Sources (Adapted and modified from Sarmah, N, et al, 2018)

Reaction Type	Bacterial Source	Industrial Application	Substrate
Hydrolysis	*B. pumilus*	Bioorganic synthesis, food and detergent industries	Long chain triglycerides
	B. licheniformis	Oil and fat industries	Trymyristin
	B. thermoleovorans	Oil and fat industries	Oil and Triglycerides
	Propionobacterium acnes	Food industries	Triolein
	P. aeruginosa	Solid waste Treatment	Phorbol 12-myristate 13-acetate
Esterification	*Acinetobacter radioresistens*	Chemical Industry	4-nitrophenyl caprylate
	B. coagulans	Food processing Industry	Oleic acid and ethanol
	B. licheniformis	Medical and Healthcare	Coumaric acid, Gallic acid
	Staphylococcus epidermidis	Flavour Industry	Fatty acid and alcohol
Transesterification	*B.subtilis*	Biodiesel	Waste cooking oil
	Burkholderia cepacia	Biodiesel, jet , fuel, light hydrocarbon oil	Jatropa oil
	Chromobacterium viscosum	Biodiesel, jet , fuel, light hydrocarbon oil	Jatropa oil

	Enterobacter aerogenes	Biodiesel, jet , fuel, light hydrocarbon oil	Jatropa oil
	Geobacillus sp.	Biodiesel production	Vegetable oil
	Pseudomonas sp.	Biodiesel	Soybean oil
Alcoholysis	*P. fluorescens*	Food industry, Cosmetics	Black current oil
	Pseudomonas sp.	Biodiesel production, quick drying oil	Triglycerides

7.2 Lipases in Production of Biodegradable Polymers

Useful biodegradable compounds can be synthesized with lipases, for example direct esterification of butanol and oleic acid produced 1-Butyloleate used in decreasing the viscosity of biodiesel for winter use. Trimethylolpropane esters are also similarly synthesized as lubricants. Aromatic polyesters have also been synthesized by lipase biocatalysts. Lipases can catalyse ester synthesis and Trans-esterification reactions in organic solvent systems which have opened up the opportunity of enzyme catalysed synthesis of biodegradable polyesters (Sirisha E., et al., 2010).

7.3 Lipases in Textile Industry

Lipases are employed in the textile industry to aid in the removal of lubricants, to increase the level of fabric absorbency for improved levelness in dyeing. Occurrence of streaks and cracks in the denim abrasion systems can also be reduced. Commercial preparations consisting of alpha amylase and lipase are being employed for resizing of the denim and other cotton fabrics, (Houde, A, et al, 2004).

In order to improve the capability of the polyester fabric to improve uptake chemical compounds (fabric finishing compositions, dyes, antistatic, anti-staining, antimicrobial, antiperspirant compounds), polyesterase are being employed.

7.4 Lipases in Detergent Industry

Use of lipases in the detergent industry represents the main significant application of lipase enzyme, it is widely used in detergent formulations to remove fat because it is optimum at 40°C; is stable in proteolytic wash solutions; shows oxidation stability; and is stable toward several other detergent ingredients including surfactants.

In the past, ground porcine or bovine pancreases, rich in lipases, were used in the fine chemical industry as detergent additives. Commercially available powder detergents and automatic dishwasher detergents usually contain one or more enzymes formulations consisting of protease, amylase, cellulase and lipase **(Houde, A, et al, 2004).** Earlier Patent (# 6,265,191, issued 07/24/2001) has been granted where lipase immobilized on surfaces used to facilitate oil removal from the surfaces and to alter wettability of the surfaces of fabric.

Table 4: Reactions Catalysed by Lipases Derived from Fungal Sources (Adapted and modified from Sarmah, N, et al, 2018)

Reaction type	Fungal Source	Application Domain	Substrate
Hydrolysis	*A. Oryzae*	Production of miso	Soybean
	A. Nidulans	Food and cosmetic industry	Medium chain and long fatty acids
	Collectotrichum gloeocporiodes	Food industry	Olive oil
	Pythium ultimum	Biocontol Agents	Plant lipids
	Penicillium chrysogenum	SL Productions	Waste cooking oil
	R. chinensis	Flavoured milk products	Milk ,fat and endogenous esters
	Yarrowialipolytica	Cosmetic industry	Castor oil
Esterification	*Colletotrichum gloeasporioides*	Food industry	Butyl butyrate
	Rhizomucor methei	Chemical Industry, cosmetics , detergents, agriculture, waste treatment	Jatropa oil Engkabang fat and oleyl alcohol
	Penicillium restrictum	Solid waste treatment	Bambasu Oil cake
	Penicillium simplicissimum	Medicinal, cosmetics, plastic additives	Lauric acid

	Geotrichum sp., *Rhizopus* sp.	Food and beauty industry	Iso amyl alcohol and Butyric acid
	R. oryzae	Wax production Chemical industry	Palm stearin
	Penicillium roqueforti	SL production	Oleic acid
Trans-esterification	*Collectotrichumgloeosporioides*	Chemical industry	Short chain alcohols
	T. lamuginosa	Biodiesel, Waste treatment	Waste oil, Rapeseed oil
	Penicillium camemberii	Bakeries, cosmetics, medicines and lubricants	Saturated monoglycerides
	P. expansum	Biodiesel production	Corn and microalgal oils
Deacytylation	*Fusarium globulosum*	Chemical industry	Peracetates of perphenolic , aromatic ketones
Alcoholysis	*R. michei*	Incorporation of different fatty acids	Tripalmitin
	R. oryzae	Detergents, cosmetics, Lubricants	Soy Phospholipids
Acidolysis	*T. languginosa*	food industry	Lard
	R. oryzae	Bilubricant and food industry	Oleic acid
	R. michei	Biodiesel, lubricants, plastic and food industry	Canola oil
Saponification	*Penicillium solitum*	Soap production	Long chain oils and fats
Ethanolysis	*R. arrhizus*	Biodiesel production	Triolein
Hydrolytic kinetic resolution	*Geotrichumcandidum*	Chemical industry	Trans-2-(4-methoxybenzyl)-1-cyclohexayl acetate
Multitude of reactions	*P. citrinum*	Waste treatment	Plam oil effluents

The lipase is isolated from a *Pseudomonas*, is immobilized on surfaces to facilitate oil removal from the surfaces and to alter hydrolyse an oil stain on dry fabric or fabric in laundering solutions. The absorbed lipase has enhanced stability to denaturation by surfactants and to heat deactivation. The other common commercial applications for detergents is in dish washing, a bleaching cleaner, contact lens cleaning, clearing of drains clogged by lipids in food processing or composition, decomposition of lipid contaminants in dry cleaning solvents, liquid leather domestic/industrial effluent treatment plants, degradation of organic wastes on the surface of exhaust pipes, toilet bowls, removal of dirt/cattle manure from domestic animals by lipases and cellulases **(Sirisha E., et al., 2010).**

7.5 Lipases in food processing, flavour development and Improving quality

Cheap oils could also be upgraded to synthesize nutritionally rich food. Fat and oil modification is one of the prime areas in food processing triacylglycerols like cocoa butter substitutes, low calories triacylglycerols, oleic acid enriched oils and for retailoring of vegetable oils. Lipases have also been used to amend food flavour by synthesis of esters of short chain fatty acids and alcohols, which are regarded as flavour and fragrance compounds (Houde, A,et al, 2004).

7.5.1 Infant formulas:

Infant formula offers a good alternative to breast milk and ideally tends to mimic human milk as much as possible. Milk fat represents the main source of energy in human milk and provides the lipids required to build the structure of cell membranes. The major triglyceride present in human milk is unsaturated at the sn-1,3 positions and saturated at the sn-2 position.Palmitic acid (C16:0) represents 20–33% of the total fatty acids with one-third located at the sn-2 position. Higher amounts of Palmitic acid reported in blood samples taken from infants fed with human milk compared with that found in infants fed with formula containing the same total concentration of Palmiticacid .During producing a monoacylglycerol with Palmitic acid at the sn-2 position that is more readily digestion, pancreatic lipases specifically hydrolyse fatty acids at the sn-1, 3 positions, absorbed than free Palmitic acid. Free Palmitic acid binds to calcium and forms poorly absorbed insoluble soaps that cause constipation (Hasan, F. et al 2006 and Houde, A.,et al, 2004).

7.5.2 Cocoa Butter:

Cocoa butter is a mixture of oil and fat composed of triglycerides possessing Palmitic acid, stearic acid, and oleic acid as the major components. Cocoa butter is a fat with a high commercial value for the confectionery industry. The high price of cocoa butter is the result of its low availability. Consequently, interesterification of abundant and less expensive fats, including illipe fat, shea butter, sal fat, and kokum butter, offers a good or stearic acids at the sn-1 and sn-3 positions by a selective lipase produces cocoa butter substitutes with a cooling, melting sensation characteristic of chocolate and similar physical properties at a lower cost (Hasan, F. et al 2006).

7.5.3 Acceleration of Cheese Ripening:

Cheese ripening is composed of a complex sequence of events and is the result of many transformation processes such as proteolysis and lipolysis in milk by indigenous micro-flora. The attributes of texture, aroma, and visual appearance characterize the different types of cheeses. Cheese texture is related to the fat content, and aroma is generated by fat degradation .The addition of exogenous lipase accelerates the ripening process. However, the addition of free lipases to the process can lead to excessive lipolysis resulting in texture and flavour defects. Lipases have also been employed in the production of leaner meat such as in fish by removal of the fat (bio-lipolysis). Lipases of microbial origin have been used for enhancing rice flavour, modifying aroma of soybean milk and accelerating the fermentation of apple wine (McKiernan et al, 2017).

7.5.4 Bakery products, confectionery and cheese flavouring:

Lipases are widely used in the dairy industry for the hydrolysis of milk fat for the purpose of flavour enhancement and ripening of cheese.The free fatty acids formed by catalysis action of lipases lend specific flavour characteristics to soft cheeses. Lipases mainly release short chain (C4 and C6) fatty acids leading to a sharp, tangy flavour, while the release of medium chain (C12, C14) fatty acids imparts a soapy taste to food (Verma N, et al, 2012).

Lipase is used in development of enzyme modified cheeses (EMC) which is being used as an ingredient in various food products (dips, sauces, dressings, soups, snacks, etc.). Lipases have been used in the improvement of flavour in coffee to form the creamy flavour, and buttery texture of toffees/caramel.

Blue cheese flavour development is due to enzymes from *Pencilliumroqueforti.* Lipases from *A. niger, R. oryzae, C.cylindracea*are used in bakery products.

7.5.5 Lipases in tea processing

The quality of black tea is dependent great extent on the dehydration, mechanical breaking and enzymatic fermentation of tea shoots. Enzymatic hydrolysis of membrane lipids during black tea manufacturing commence the formation of volatile products with characteristic flavouring properties. Enhancement in PUFA and reduction in total lipid content was observed by lipase produced by *Rhizomucor miehei* (Verma N, et al, 2012).

7.5.6 Lipases in cosmetic Industry

Immobilized *Rhizomucor meihei* lipase was used as a biocatalyst by Unichem International (Spain) in the production of isopropyl myristate, isopropyl palmitate and 2-ethylhexylpalmitate to be used as an emollient in personal care products such as skin and sun-tan creams, bath oils etc. The enzyme in place of the conventional acid catalyst gives increased product quality requiring minimum downstream refining (Verma N, et al, 2012 and Houde, A. et al, 2004). Immobilized lipase was also employed to prepare water-soluble retinol derivatives which find extensive use in skin care products.

Table 5: Reactions Catalysed by Lipases Derived From Yeast Sources (Adapted and modified from Sarmah, N, et al, 2018)

Reaction Type	Yeast Source	Industrial applications	Substrate
Hydrolysis	*C. rugosa*	Oil plants,varnishes	Linolenic acid
	T. lanuginosus	Lubricants and food industry	Soybean oil
	C. antartica	Synthesis of valuable fatty acids	Olive oil
	C. rugosa	Perfume industry	(S)-3-(2-methlyfuryl) thioacetate.
Esterification	*C. antartica*	Food,cosmetics and	Lactic acid

		pharmaceuticals	
		Chemical ,cosmetics and detergents	Dihydoxy stearic acid
Transesterification	*C. antartica*	Biodiesel	Soybean oil
	Chromobacterium viscosum	Biodiesel, jet fuel	Jatropha oil
	C. antartica	Waste treatment ,polymer industry	Waste oil, Trimethylolpropane
Alcoholics	*C. antartica*	Pharmaceuticals, food industry	Acyl Ribonucleosides
Enantioselective hydrolysis	*C. rugosa*	Cosmetics and detergents	Butter oil
Acidolysis	*C. antartica*	Pharmaceuticals , food and cosmetics	Acyl glycerol, borage oil

7.6 Medical Applications of Lipases

Lipases isolated from the wax moth (*Galleria mellonella*) showed bacteriocidal action on *Mycobacterium tuberculosis* (MBT) H37Rv. Lipases can be used in potential treatment of malignant tumors as they are the activators of Tumor Necrosis Factor. Also, human gastric lipase (HGL) constitutes a good candidate tool for enzyme substitution therapy. Which can be used in gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. (McKiernan et al., 2017).

Berrobi et al. have filed a patent on Lipase with hyaluronidase and/or thiomucase enzyme formulation which can be used for the treatment of skin inflammations. Lipase from *Candida rugosa* has been employed to synthesize lovastatin, a drug that lower serum cholesterol level.

7.7 Lipases in Leather Industry

Lipases represent a more environmentally sound method of removing fat. Processing of fatty raw materials including animal skin/hides require degreasing by conventional or enzyme based methods. Conventional methods can give rise to environmental problems such as volatile organic compound (VOC) emissions. Whereas, lipase (alkaline stable and acid active) can be used to treat skin/hides and providing more uniform colour and a cleaner appearance. Better quality product can be obtained using lipase when compared with traditional methods. Lipase from *Rhizopus nodosus* was employed for the degreasing of wooled sheep skins (McKiernan et al., 2017).

7.8 Lipases in Pharmaceutical Industry/ in resolution of racemic mixtures

Lipases can be used to resolve the racemic mixtures and to synthesize the chiral building blocks for pharmaceuticals. Some lipases employ stereospecific hydrolysis of esters and thus help in resolution of racemic mixtures. Lipases are currently being used by many pharmaceutical companies world-wide for the preparation of optically active intermediates on a kilogram scale. Lipase mediated resolution is being used in biotransformations by biotechnological companies, such as Enzymatix in the UK etc. A Regioselective modification of poly-functional organic compounds is one of the potential applications of this enzyme. Lipases have been effectively useful in the regioselective modification of castanospermine, a promising drug for the treatment of AIDS.

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