A SYSTEMATIC OVERVIEW ON THE UPSTREAMING, DOWNSTREAMING AND INDUSTRIAL APPLICATIONS OF MICROBIAL LIPASES

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ABSTRACT

Lipases belong to the class hydrolases, and act as biocatalysts. Producer organisms are plant, animals (particularly mammals) and microbes (preferred over other potential sources). Lipase is a high value compound due to high productivity rate. Thus, exhibit wide range of applications in different industries such as food (dairy, fat, and oil, beverages, bakery), detergent, paper and pulp, biodiesel production, pesticides, and pharmaceuticals. In addition, these are used in bioremediation of different effluents. Lipases are unique in the sense. Two techniques were highly used for production of microbial lipases e.g., solid-state, and submerged fermentation. But solid-state fermentation is preferred over submerged fermentation due to high productivity. Enzyme immobilization has been discussed briefly. Purification depends upon the nature of lipase and done by ammonium sulphate precipitation, ultrafiltration, and extraction by solvents like acetone and ethanol. This review focuses on the information which has been round up over the last two decades on sources of lipase, production, immobilization, unique purification techniques as well as the wide spectrum of industrial applications.

Keywords: Lipase, SSF, SmF, purification, applications, immobilization.

INTRODUCTION

Lipases are hydrolases (EC 3.1.1.3) that have ability to catalyze lipid (triacylglycerols) in fatty-acids and the glycerol (Fig. 1). It is also involved in catalysis of transesterification and hydrolysis of other esters (Treichel *et al*., 2010). Naturally, Triglyceride's hydrolysis gives diglycerides, monoglycerides, fatty acids and glycerol by using the lipases. They have ability to reverse the hydrolysis of the fats in the non-aquatic medium and copiously present in the nature (Ilesanmi *et al*., 2020). Lipases are universal enzymes of substantial physiological importance. Compared to esterases, activation of lipases occurs when absorbed to oil-water interface (Sharma *et al*., 2001). Lipases function in aqueous environments on the carboxyl ester bond, existing in the triacylglycerols to release glycerol and fatty acids. In micro-aqueous environment, lipases have distinctive capability to reverse reaction which leads to the esterification, acidolysis and alcoholysis (Adetunjii and Olaniran, 2021). Catalytic potential of the lipases has been enhanced and selective through unique process of the solvent engineering, molecular imprinting, and molecular methods such as direct evolution and protein engineering (Jaegar and Reetz, 1999). Lipase reported as monomeric protein and their molecular weight lies in between 19-60 kDa. Lipases exhibited pH dependent activities, usually at pH 7-4 (Chandra *et al.*, 2020). They are the serine hydrolases, that act on the interface of lipid and water (Patel *et al*., 2021). The catalytic triad of lipase is constituted of the Asp/Glu-His and generally Gly-X-Ser-Gly sequence (consensus sequence) is located round the active site of the serine. 3D structures of the lipases show property of α/β-hydrolase fold (Nardini and Dijkstra, 1999).

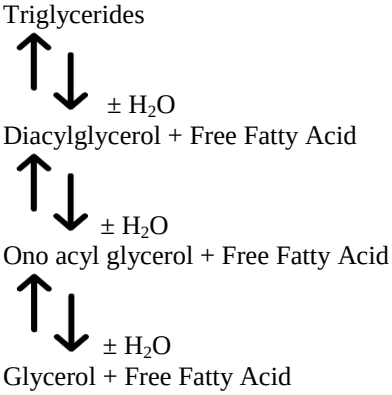

Fig.1 Schematic representation of the lipase reaction.

Lipase had been appeared as chief biocatalysts providing billion dollars, used in lipid technology, and utilized in the in-situ metabolism of lipid and ex-situ different industrial appliances (Joseph *et al*., 2008). Eukaryotic lipases involved in numerous phases of the lipid metabolism for example digestion of fat, absorption, reconstitution, and the metabolism of lipoprotein. Lipases in plants are present in the tissues that are energy reserved (Balashev *et al*., 2001). Based on positional hydrolysis on the triacylglyceride (TAG) molecule, microbial lipases are divided into two groups. First group are nonspecific that cause the full hydrolysis of the TAG to Free fatty acids (FFAs) and glycerol. That comprises lipase from *Staphylococcus aureus, Geotrichum candidum, Corynebacterium acnes, Chromobacterium viscosu* and *Penicillium cyclopium.* Second group cause hydrolysis of fatty acid that esterified in the Sn^{-1} and Sn^{-3} sites, that result into diacylglycerol and one fatty acid or monoacylglycerol and two fatty acids (Kilara, 2011).

Due to their varied range of significance, lipases persist the topic of the rigorous study. Its research has focussed mainly on the structural characterization, explanation of method of the action, sequencing, cloning of the genes of lipase and the characterization of the performance (Bornscheuer, 2002). Lately, lipases appeared as important enzymes in the biotechnology due to versatile properties and have use in wide range of the industrial appliances e.g., in food technology, detergent or biomedical sciences (Jaegar *et al*., 1998). Lipases have application in the processing of organic chemical, in detergent preparation, biosurfactants synthesis, oleochemical industry, in agrochemical industry, in diary industry, paper, cosmetics, in pharmaceutical processing and nutrition. Development of the lipase founded technologies, aimed the production of the unique compounds that is quickly intensifying the usage of that enzymes. Large amount of the lipases with appropriate properties are available and the attempts have been happening to the commercialization of biotransformation and production founded on the lipases (Liese *et al*., 2000).

SOURCES OF LIPASE

Lipases are abundant in the nature and yielded by many plant, animal, and different microbes. Microbial lipases demonstrate extensively operating class of the enzymes in the biotechnological, industrial, and the organic chemistry practices (Sharma *et al*., 2001).

Filamentous Fungi

Fungi are capable to produce lipase in many habitats (seeds, treated vegetable oils, contaminated soil through oils, impaired food, and dairy products) (Singh and Mukhopadhyay, 2012).There are some fungal genera, which are important source of lipase production for example *Rhizopus*, *Aspergillus*, *Penicillium*, *Geotrichum*, *Mucor*, and *Rhizomucor*. Lipase productions differ according to fungal strain and conditions used, such as the composition of the growth medium, production conditions, pH, and temperature of the medium, and carbon and nitrogen as other sources. Many scientists discovered 59 lipase-producing fungal strains from Brazilian savanna soil with the help of the enrichment techniques (Colen *et al*., 2006). Lipase production with the help of *G. candidum* was also investigated in the shake flask and bench scale bioreactor with the help of different inducers (Maldonado *et al*., 2012).

Yeast

Conferring to the Vakhlu and Kour (2006), vital telluric species of the yeasts which had been discovered to yield lipases are *Candida rugosa, Saccharomycopsis crataegenesis, C. tropicalis, C. antarctica,* Pichia *burtonii, Trichosporon asteroids, C. parapsilopsis, C. deformans, Rhodotorula pilimornae, C. cylindracea, C. valida, Yarrowia lipolytica, Rhodotorula glutinis, Pichia xylosa, Torulaspora globosa,* and the. Genes which translate lipase in the *Candida sp*., *Geotrichum sp*., *Trichosporon sp*., and *Y. lipolytica* were clonover-expressed in 2007 (Wang *et al*., 2007). However, lipases from the *C. antarctica* and the *C. rugosa* widely used in various fields, numerous publications describing the lipases production by further yeasts in Table 1.

Bacteria

The significant bacterial genera are *Achromobacter, Alcaligenes, Arthrobacter, Bcacillus, Burkholderia, Chromobacterium* and *Pseudomonas*. Bacterial lipases have been greatly affected by many parameters and nutritional environment for example carbon, lipids, inorganic salts, and nitrogen (Liew *et al*., 2015). The genera *Burkholderia* and *Pseudomonas* due to their high enzyme activity are greatly used to produce lipase at low cost, in this way waste disposal problem was solved (Bose and Keharia, 2013). Bulk production of bacterial lipases is easier than others and commercially very important extracellular enzymes (Jaegar *et al.,* 1994).

Table 1. Microorganisms as protentional lipase producers.

Microorganism	Source	Reference
Acinetobacter sp.	Bacteria	Barbaro *et al*. (2001)
Alcaligenes sp.	Bacteria	Mitsuda *et al*. (1988)
B. subtilis	Bacteria	Jaegar and Reetz (1999)
Propionibacterium acnes	Bacteria	Jaegar *et al.* (1994)
S. aureus	Bacteria	Simmons *et al*. (1996)
V. chloreae	Bacteria	Jaegar *et al*. (1998)
Rhizopus arrhizus	Fungi	Tan and Yin (2005)
Rhizopus chinensis	Fungi	Wang *et al*. (2007)
Penicillium citrinum	Fungi	D'Annibale *et al*. (2006)
Geotrichum candidum	Fungi	Ramos *et al*. (2015)
Candida utilis	Yeast	Grbaycic *et al*. (2007)
Trichosporon ashi	Yeast	Kumar and Gupta (2008)
Candida rugosa	Yeast	Rajendran *et al*. (2008)
Saccharomyces cerevisiae	Yeast	Ciafardini *et al*. (2006)

SUBSTRATES

Microbial lipases have been generally extracellular, and its yield has been significantly affected by the composition of medium besides physico-chemical factor like pH, dissolved oxygen, and temperature. For lipase activity expression, carbon source has been studied as major factor. These are mostly produced in lipid presence for example oil or inducer e.g., fatty acids, triacylglycerols, hydrolysable ester, glycerol, Tweens, and bile salt (Sharma *et al*., 2001).

Synthetic Medium

Typically, great yield has been obtained by the optimization of culture medium. Different scientist used different synthetic media to produce lipases such as: In 1995, Lin and his colleagues observed that 0.5% sodium nitrate, 5% glycerol and the 0.1% of thiamine offered superlative results (54 Uml^{-1}) (Lin *et al.,* 1995). Gupta *et al.* (2007) used synthetic medium, comprise of 0.1% glucose, 3.0% olive oil, 0.5% NH_4Cl, 0.36% yeast extract, 0.1% K_2HPO_4, 0.01 g $MgCl_2$ and 0.4 mM $CaCl_2$, that increased the yield up to 12-fold. In 2008, Wang and his colleagues, optimized the fermentation media for the yield of lipase by the *R. chinensis*. Optimized media for the enhanced lipase activity comprised of peptone, maltose, olive oil, $MgSO_4.7H_2O$ and K_2HPO_4. Abada (2008) yielded lipase from the *B. stearothermophilus*. Abada examined that the usage of the xylose, alanine, tryptophan, potassium nitrate and phenylalanine as additional for the highest production of lipase.

Agro-industrial Residues

Agro-industrial residues have been focused for the fermentative process because of their potential benefits. Use of agro-industrial residues delivers alternate substrates and help to solve the pollution problems, that might be produced by their discard. Substrate nature is the important factor that affects the fermentation process. Thus, optimization process involved screening of numerous agro-industrial wastes (Treichel *et al*., 2010). In 2007, Good outcomes had been observed with the melon solid waste that is supplemented with the 1% olive oil and NH_4NO_3. Mala and his colleagues (2007) developed solid-state fermentation for lipase yield using *Aspergillus niger* strain (MTCC 2594) through oil cake and wheat bran respectively, and result indicate the supplement of the gingelly oil cake with the wheat bran enhanced activity of lipase up to 36% and the activity had been 384 $Ugds^{-1}$ (Mala *et al.*, 2007). Kempka *et al*. (2008) observed lipase from the *P. verrucosum* by the solid-state fermentation with soybean meal, liquor of corn steep, molasses of sugar cane, yeast hydrolyzed, yeast extract, soybean oil, peptone, olive oil, castor oil and sodium chloride. Soy-bean meal gave good results.

FERMENTATION

Lipase is produced by two type of fermentation.

Submerged fermentation

Improvement in the submerged fermentation environments such as C/N ratio, nitrogen source, carbon source, tank volume, temperature and inducers are important to enhance the lipase production. Submerged fermentation processes are mainly use in the industrial large-scale production of the enzyme, SmF process are easy to control and monitor (Silveira *et al*., 2016). The quantitative comparison among the SmF and SSF is difficult because of their

different methods that are employed for determination of the lipase activity (Treichel *et al*., 2010). In 2011, Narasimha *et al*. (2011) estimated the production of lipase from the bacterial strain such as *pseudomonas sp*. by submerged fermentation with various sources of carbon and concluded that lipase production was increased when 'C' source in medium was olive oil. Basheer and their coworker (2011) isolated and identified *Aspergillus awamori* that produce extracellular lipase inside sea water. Cultivation of *A. awamori* with submerged fermentation demonstrate that the enzyme production start after 36 h of incubation, which attained maximum at the incubation of 96 h that was 495 U/ml, however at 108 h of incubation, maximum enzyme activity was noted that was 116.6 U/mg protein (Basheer *et al*., 2011). In 2012, Shakila Begam used defatted and non-deffated soybean flour for lipase yield from the bacterial strain of *Serratia marcescens*. By usage of defatted soybean flour, large amount of lipase yields up to 28 U/ml had been attained in SmF (Begam *et al*., 2012).

Solid State Fermentation

In SSF, solid matrix has been utilized and occurred in absence or almost absence of the free water. Therefore, substrate requires moisture and source of the nutrient for the development of microorganism. Sarkar and Laha (2013) attained that *A. niger* lipase activity by SSF was 4.8 IU/ml that was 3-folds higher than SmF (1.5 IU/ml), indicating that lipase founded by the solid-state fermentation was concentrated than SmF lipase. Furthermore, in 2015, Martindel campo and his colleagues proposed that SSF had been an outstanding system for production of lipase/esterase activity from *Natronococcus sp*. a halophilic archaeon (Martindel *et al.,* 2015). In SSF conditions, the lipase yield was reached to 29.2 U/l that was greater (6.3-fold) then submerged fermentation conditions. Comparably, the lipase activity with the p-nitro-phenyl butyrate was 388 U/W in the submerged fermentation, that was almost 2.5-fold fewer than lipase activity in solid state fermentation. Raw material such as industrial waste of palm oil had been utilized for lipase yield by *Aspergillus niger* and SSF showed best results. Within solid state fermentation, maximum activity of lipase was observed after cultivation of 72 h was 77 IU/g of the dry substrate (Silveira *et al*., 2016).

PURIFICATION STRATEGIES

Many commercial uses of the enzymes do not require homogenized preparation of enzyme. Still, degree of the purity has been required, that depend on final uses in the industries. Moreover, enzyme purification is essential for the understanding of 3-dimensional structure or relationship between the structure and function of proteins. For commercial purposes, purification strategies could be inexpensive, excessive yielding, rapid and acquiescent to the big scale setups (Gupta *et al*., 2004).

Concentration

Concentration techniques for extraction and the purification of lipase include precipitation of ammonium sulfate, ultrafiltration, and extraction through organic solvents for example acetone and cold ethanol. Methods selections depend upon the properties of lipase, constitution of the extract and process cost. Precipitation is mainly important technique. It is used to modify the solvent through addition of salts to change protein solubility and favor protein aggregates formation (Melani *et al*., 2019). Ayaz and his colleagues in 2015 used 80% ammonium-sulfate precipitation and chromatography (via gel filtration) for purification of alkaline extracellular lipase found from the *Streptomyces sp*. that caused purification up to 5.52-fold, that had 68.05 U/mg activity (Ayaz *et al*., 2015). This study reveals that the concentration techniques with the chromatographic procedures gave better results.

Precipitation of protein through organic solvents should be done at temperatures near to 0℃ for the prevention of denaturation of protein and to preserve the biological properties. In 2014, Patel investigated solvent-stable extracellular alkaline lipase enzyme from the DMVR46 specie of *Pseudomonas*, which determined stability in the polar organic solvents for example cyclohexane, n-hexane and isooctane retaining above 70 percent of lipase original activity (Patel *et al*., 2014). Ultrafiltration comprises another form of the concentration method, which determined particle size separation. Membranes of ultrafiltration are illustrated by molecular weight cut-off, which has been described through species having negligible molecular weight for that membrane showing 90% rejection. Ultrafiltration combined with various extraction methods give good performance in purification process (Charcoss *et al.,* 2011).

Chromatography

It is a technique which is used to separate different but alike solutes with great resolution. While costly, it is also widely used separation method in the field of biotechnology. Chromatography is founded on variance dispersal of the solutes by the biomolecules in the mobile phase transferred by stationary phase enclosed in column. That column identified as the chromatographic matrices, mainly consist of the hydrophilic materials which establish no

collaboration with biomolecules (Sun *et al*., 2011). Most used method is ion-exchange chromatography. According to Incharoensakdi and Sivaramakrishnan, lipase purification take place through ion-exchange chromatography and precipitation of ammonium sulfate. The fraction of ammonium sulphate (precipitated) was employed to CL-4B column of phenylsepharose and the pre-equilibrated through 250 mM potassium phosphate buffer having pH 7.0. Enzyme purified up to 5.1-fold with 10.5% yield (Sivaramakrishnan and Incharoensakdi, 2016). HIC (Hydrophobic interaction chromatography) has alternative utilized technique. Objective of HIC based on the separation and purification of biomolecules via hydrophobic collaboration with the hydrophobic ligands combined to the porous medium. Stationary phase of the HIC has weak ligands like short chain alkyl and phenyl immobilized on hydrophobic matrix (Sun *et al*., 2011).

Aqueous two-phase systems (ATPS)

Immiscible two aqueous phases of the ATPS have been considered as liquid purification process for concentration, separation and biomolecule extraction by high simplicity, productivity, less time, scalability, versatility, and efficacy of the system (Show *et al*., 2012). The ATPS contain the mixture of the salts and polymers. The polymers could be poly-ethylene glycol, dextran, and the polypropylene glycol. The salts could be sulfates, phosphates, ionic liquid, and surfactants. Low-molecular- weight alcohols can be present such as propanol and ethanol. An acute concentration, that permits formations of two-aqueous phases required for every constituent. ATPS is generally used for purification of the proteins and separation of proteins from the cell debris. This technique is classified in five key groups such as polymer-polymer, alcohol-salt, micellar and the ionic liquid-founded systems (Benavides *et al*., 2011). PEG + dextran ATPS are example of polymer-polymer ATPS which are non-toxic and categorized as safe for the recovery of therapeutic proteins at the industrial scale. PEG + dextran ATPS has been used for purification of lipase from bacteria e.g., *Burkholderia pseudomallei* (Ooi *et al*., 2011). Alizadeh and Khayati in 2013 examined Rhodotorula glutinis fermentation conditions in ATPS including salts and PEG. Fermentation at 24℃ and 6.6 pH with 12.5% oxalate potassium, 17.5% PEG 4000, fermented broth demonstrates as best system, having 13.9 purification factor and 71.2% enzyme yield in upper PEG rich phase.

Reverse Micellar System

It is a method in which extraction of liquid-liquid occur that use organic solvent comprising water drops steadied by the layer of the surfactant particles. Reverse micellar technique suggests numerous advantages like relieve of the scaleup, constant mode of the operation, low-interfacial tension (Nandini and Rastogi, 2009). Meanwhile, insolubility of lipase production and substrate delay the manipulation and characterization of lipase in aqueous, reversed micellar technique appears as probable solution intended for the barriers stemming through heterogeneity of the media. Another scientist studied *Rhizopus chinensis* and filamentous fungus, which determine the capability to yield mycelium-based lipase for manufacture of the ester into non-aqueous mixtures. It has described that the mycelial aggregation through fungi growth that led to further production related to free growth of the cell (Melani *et al*., 2019).

IMMOBILIZATION

Natural and the immobilized lipases have been accessible commercially. Immobilization is a process that enhances the recyclability of the pricey lipases. Similarly, immobilization could improve the stability of enzyme and activity. For immobilization of lipases, there are many methods such as precipitation or adsorption onto the hydrophobic materials, entrapment in the polymer gels, covalent attachment with functional groups, adsorption in the macro-porous anion substitute resins, sol-gel entrapment, and micro-encapsulation in the lipid vesicles. Both types of *G. candidum* lipases had been immobilized through porous polypropylene supports, pre-coated with the oval albumin to improve the constancy in the organic solvents at higher temperatures (Sharma *et al*., 2001). For the immobilization of lipases through adsorption on the hydrophobic membranes, polymer membranes load more lipases than hydrophilic membranes, which usually seem than enzyme that absorbed by hydro-phobic membrane. Usage of fat membrane and vacant fiber reactors to the bio-transformations with the immobilized lipases had described widely (Bouwer *et al*., 1997). Lipase of *C. cylindracea* was immobilized with benzene co-polymer of methyl acrylate divinyl and their derivatives. Immobilized lipases contain enhanced resistance to the thermal denaturation (Xu *et al*., 2000).

Arroyo immobilized covalently the lipase B of *C. Antarctica* on sepharose, silica and alumina. It increases the solubility of catalyst and then improves its mode of the deactivation comparative to native enzyme. Hiol *et al*. (2000) used amberlite IRC50 for the immobilization of the purified lipase of *Rhizopus oryzae*. Associated to other assistance, Amberlite proposed great adsorption capacity or also offers long term constancy of the immobilized lipase. Constancy of the immobilized enzymes were evaluated by examining its capability to esterify hexanol and

oleic acid at 30℃ in the cyclohexane. Repeated usage of immobilized lipase through period of the 3 weeks decreased its 18% esterifying capacity. Over same period, lipase hydrolyzing activity decreased by the 80%.

APPLICATIONS

Applications of lipase in food and dairy industry

Food industry

Food is made up of fats and oil. The nutritional value of triglycerides is greatly affected by numerous parameters such as length of fatty acids, unsaturation, and position of fatty acids. We can adapt characteristics of lipid or fat by changing the position of fatty acids chain and replaced the previous one with recent ones. According to this way with little cost, less desirable lipid could be changed into desirable and high nutritional value fat. Cocca butter which has made up of the palmitic and the stearic acids and a high value fat, having a melting point of 37°C. On melting it produce cooling effect in mouth for example chocolate. Technology based on lipase involve in mixed hydrolysis reaction synthesis has been utilized to changed less desired fats into high value fat to the cocoa butter alternatives (Sharma *et al*., 2001).

Tea processing

Camellia sinensis is a tea plant that is being prepared commercially from bud and apical two leaves of the plant. Development of volatile products started when membrane lipids break with help of the enzyme during the synthesis of black tea (Girelli *et al*., 2020).

Black tea with flavor properties makes the importance of lipid in flavor enhancement. The standard of black tea depends upon dryness, and fermentation through enzymes to which tea leaves are exposed. When total lipid content reduces, that detect the level of polyunsaturated fatty acids which enhanced by *Rhizomucor miehei* lipase (Clough, 2011).

Dairy industry

Lipases are extensively utilized in the dairy industry for the hydrolysis of milk fat, to change the chain length of fatty acid and to improve the flavor of cheese. Milk fat after the action of lipases on many products particularly soft cheeses with flavor properties produced with free fatty acids (Hamdy *et al*., 2017). Enzyme Modified Cheese (EMC) is synthesizing when cheese incubated at high temperature with enzyme and lipase catalysis is being used to harvest concentrated flavor using lipase (Law ,1999). In EMC, the concentration of fat is 10 times greater than the normal cheese and used as an ingredient in many other industrial food products (Chandan, 2008).

Biosensors in food industry

Lipases are used as biosensor for the quantitative determination of fatty acid such as triacylglycerol due to their high efficiency. Lipases are important in the food industry particularly in fats and oils, soft drinks, drug industries, beverages, and in medical diagnosis (Zehani *et al*., 2014). Triacylglycerol breakdowns into the glycerol through using the biosensor as lipase enzyme (Despres *et al*., 2009).

Bakery products, cheese flavoring and confectionery

In the dairy industry, lipases are extensively used for the milk fat hydrolysis. Recently, cheese flavor can be improved by using lipase enzyme. The cheese ripening hastening, and manufacturing of cheese like products such as the butterfat, cream lipolysis is being done by using lipases. Many dairy products produced from free fatty acids by the action of lipases with their specific flavor features such as the soft cheeses (Jooyandeh *et al*., 2009).

Applications of lipase in pharmaceutical and medical industry

Pharmaceuticals

Some lipases have been used in pharmaceutical companies for their therapeutic benefits such as it has been applicable for curing of the skin scalp diseases and hair loss (Baldo *et al*., 2011). Many drugs are in the process of clinical trials for treatments of different diseases such as cardiovascular, obesity, anxiety, inflammation, and pain (Nomura *et al*., 2016). For detection of the tuberculosis (TB), lipase could be applicable for diagnostic purposes. Mycobacterium tuberculosis lipase has been used to check TB infection with high sensitivity and specificity detection (Brust *et al*., 2011). Specific lipase level in the blood serum has been used for detection of the acute pancreatitis (Suzuki *et al*., 2014). Drug lovastatin manufactured from *C. rugose* lipase are also used to reduce serum cholesterol level (Tietz and Shuey, 1993). Lipases are applicable for the synthesis of organic compound and optically active acids, alcohols, and esters. These enzymes are used in organic preparation of compounds which are optically active e.g., alcohols, amines, and carboxylic acids. It can synthesize a compound named as Polix which is

used in for the preparation of anticancer drug especially in ovarian cancer. It is also used in nanoparticles technology for biosensor development (Melani *et al*., 2019).

Diagnostic tool

In medical field, lipases have been used as important marker enzymes and drug targets. Lipase could indicate specific infection/disease and could be applicable as diagnostic tool. Consequently, it analyzed serum triglycerides using enzyme inter-connected colorimetric reaction (Wenk, 2005). Lipase in the blood stream has been used as diagnostic tool for the detection of acute pancreatitis (Vissers *et al*., 1999). Some pathogenic bacteria for example *S. aureus*, *P. acnes* and *C. acnes* lipases has initiated their influence on the skin rash in the acne patients (Dougherty *et al*., 1991).

Applications of lipases in environment

Leather degreasing

In degreasing technique, solvents and emulsifiers or their mixture used to obtain required quality of product in leather industry. Solvents and surfactant usage is very common for sheepskins. The liquid wastes of leather industry waste consisting of large amounts of both solvents and emulsifiers are harmful with other chemical for surrounding environment. Currently, new enzyme preparations useful in leather production have made new chances for enzymatic applications in the leather industry (Choudhary *et al*., 2000). Skin and hides contain moderate fat amount and grease removes via lipase enzymes. Lipases hydrolyzed triglyceride to glycerol and free fatty acids and helps in the isolation of collagen (Fickers *et al*., 2005).

Detergent industry

Lipases have been used as essences in the laundry and the household detergents in industry, due to capable of hydrolyzing fats. Lipases in detergents are especially chosen to fulfill these necessities: (1) little specificity of substrate; (2) capacity to endure hard washing situations (such as pH 10–11 and temperature from 30°C–60°C), (3) capacity to resist dangerous surfactants and the enzymes (for example linear-alkyl benzene sulfonates and the proteases), they are pompous elements of numerous detergent formulas (Wang *et al*., 2008).

Bioconversions in organic media

Enzymes without free aqueous phase in organic media are known to show beneficial unusual properties and this developed non-aqueous enzyme systems for production and bio-transformations (Klibanov, 1997).

Pulp and paper industry

Pitch creates the severe problem in manufacturing of paper and pulp. Pitch is also called as triglycerides and waxes, and these are hydrophobic components of wood. In paper making industry, lipases are being used as removing agent for pitch in pulp production. *Candida rugose* fungal lipase has been used in Japan (Nippon paper Industries) for pitch control in paper and pulp making, which can 90% hydrolyze wood triglycerides (Jaeger *et al.,* 1998).

Biodiesel

Lipases have been used in biodiesel production after modification of fats and oils (Silveira *et al*., 2016). In the presence of aqueous medium, lipases are also able to synthesize biodiesel which is useful strategy for biodiesel production using waste oils (Melani *et al*., 2019). After enzymatic catalysis glycerol is produced, this is only disadvantage in production of biodiesel. However, several studies on biodiesel focusing biodiesel production like biofuel which keeps the glycerol in monoglyceride form (Calero *et al*., 2014). Microbial lipases that are used to produce biodiesel are produced from *A. niger*, *C. Antarctica*, *C. rugose*, *C. viscosum*, *M. miehei*, *L. plantarum*, *P. fluorescens*, *R. oryzae*, *B. cepacia* and *B. subtilis* (Stefanovic *et al*., 2018).

Bioremediation

Waste disposal and environmental protection is a major concern now a day, which leads to discovery of new bioremediation techniques. In this scenario, different enzymes like lipases, esterase and proteases which can hydrolyze ester bond is good solution. Lipases have ability to increase the bioremediation of sticky effluents e.g., fats, oils, protein waste by different industries, restaurants, slaughterhouses, hospitals (Basheer *et al*., 2011). Moreover, lipases due to their substrate selectivity are widely used in reaction of organic synthesis (Melani *et al*., 2019).

Table 2. Industrial applications of lipases.

Industry	Action	Applications	Reference
Biodiesel	Synthesis	Biofuel	Calero *et al*. (2014)
Leather	Hydrolysis	Leather products	Choudhary *et al*. (2000)
Detergents	Hydrolysis of fats	From fabrics removal of oil stains	Wang *et al*. (2008)
Paper	Hydrolysis	Better quality paper products	Jaegar and Reetz (1999)
Fats and oils	Transesterification	fatty acids, margarine, cocca butter, glycerol's	Sharma *et al*. (2001)
Dairy Foods	Hydrolysis of fat milk, cheese ripening, modification of butter fat	As flavoring agents in milk, cheese, and butter	Hamdy *et al*. (2017)
Bakery	Flavor enhancement	Shelf-life continuation	Jooyandeh *et al*. (2009)
Pharmaceutical	Hydrolysis, transesterification	Digestive aids,	Nomura *et al*. (2016)

CONCLUDING REMARKS

Lipases are one of the frequently used and produced enzymes which proved after the comprehensive analysis of literature. Due to the extensive variety of the lipases, they have isolated, purified, characterized from varied microbial sources, all commercial application demand certain exceptional features such as substrate specificity, alkaline stability, thermal stability, cold active nature, and organic solvent tolerance. This Review shows that many researchers across worldwide are trying to screen more useful lipase producing microorganisms, production techniques, optimization of media composition and other variables. Lipases are being produced with improved properties through different techniques. Processing based on lipases has a promising future, but progress rate is slow. All these attempts are justified by the uniqueness and versatility of lipase applications.

Acknowledgements: The authors acknowledge faculty of life sciences and vice chancellor for any kind of assistance provided by them.

Conflict of interest: The authors declare that there is no conflict of interest.

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(*Accepted for publication March 2022*)