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Review Article

Current Trends in the Production, Purification and Potential Applications of Microbial Lipases

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Abstract

Microbial lipases enzymes are the most widely used class of enzyme that has broad functions in industries such as textiles industry, detergent, food industry, medical and diagnosis field, cosmetics, biodegrading paper industries and biodiesel production. Microbial lipases breakdown triglycerides into fatty acids and glycerol subunits. They are very substrate specific and have an ability to perform function at high temperature and pH. There are also microbial lipases which are solvent tolerant. Although microbial lipases can be produced by both plants and animals, microbes are preferred because they are easy to cultivate and maintain and it is easy to purify enzyme from these microbes instead of complex cells of eukaryotes. The properties of these enzymes have been continuously developed by manipulating the cells by modifying culture conditions and by improving the enzyme activity by various mechanical and chemical immobilization techniques and their function are being broadened. In this review article, it has been attempted to study the recent trends in production, purification and the potential applications of enzyme along with the recent advances.

Keywords: Lipases sources; Microbial lipases; Lipases production; Lipases purification immobilization

Introduction

Microbial lipases belong to α/β-hydrolase protein family. Their molecular weight can vary from as small as 20 kDa to as large as 60 kDa. Generally, their active site consists of Ser-His-Asp. While, Glu may replace Asp in lipases from *Geotrichum candidum* lipases (Mala and Takeuchi 2008). When detected, these amino acids are arranged in Ser – Asp-His order, and residue of Ser are present in a conserved region of Gly-X-Ser-X-Gly. This conserved peptide structure of five amino acids is crucial in serine hydrolytic catalytic activity and present in all enzymes of this group of lipases. All lipases are secondary structure; however, the main difference arises from the number of α-helices and how they are arranged in the protein structure. Secondary structure also depends on the angle of folds and their number in the β-strands (Yao *et al.* 2021). Microbial lipases are also region-selective, enantio-selective and chemo-specific for their substrate (Adetunji and Olaniran 2021). Many lipases also feature lid structure, an unstable domain for permitting interaction between the specific substrate only such as oils and the enzymes. The catalytic mechanism of the microbial lipases depends on the similar catalytic triad that has been observed in serine hydrolases. This mechanism of catalyzing substrate is divided into four steps first, hydrogen is removed from the serine hydroxyl group by histidine residue at the catalytic triad and oxygen with negative charge and a carbonyl carbon with a positive charge interact through nucleophilic interaction. Secondly, oxygen anion hole is developed, which is the lipase electrophilic region and both substrate and enzyme undergo tetrahedral transition state. Thirdly, the ester bonds break generating acyl covalent intermediate complexes after fatty-acid alcohol is released. Fourthly and finally, the acyl substrate is released after temporary bond between enzyme and substrate are relieved as shown in Fig. 1 (Yao *et al.* 2021).

Microorganisms are preferred to produce lipases because they can be easily exploited for higher yield, greater thermal stability and improve activity. Microbial lipases have application in the fields such as food industry, paper industry, textile industry, medical and diagnostic field, cosmetic, biodiesel production and bioremediation. This review will strive to discuss all major aspect of the microbial lipases (Vanleeuw *et al.* 2019).

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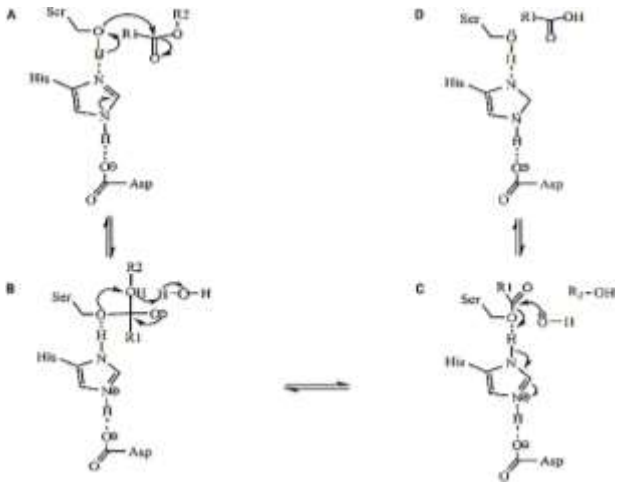

Fig. 1: The catalytic mechanism of lipases (Yao *et al.* 2021) **a)** The enzyme binds to the substrate; **b)** intermediate tetrahedral structures are formed; **c)** acyl covalent intermediate complexes are formed; **d)** deacylation

Sources of Lipases

Sources of microbial lipases

Lipases can originate from plants, animals and microbes, however lipases from microorganisms are preferred to the one from animals or plants for commercial use. They can be easily produced in bulk quantity and have little adverse effect on the environment. Most common classes of microbes are fungi, bacteria and yeast which are used for production of lipases and various species are being studied for increased production of lipases. Moreover microbes are a source of solvent tolerant as reported by Sardessai and Bhosle (2004) and temperature resistant lipases. Microbial lipases can be produced both extracellular and intracellular. The lipase from extracellular producer microbes has been studied mostly because of easy extraction and purification. The lipase from different environmental species of microbes has been studied as extremophilic lipase, purified easily as well as has greater stability and can be modified desirably (Ali *et al*. 2023).

Fungal lipases: Fungi are the principal source of lipases in contemporary enzyme production due to high stability under extreme physical and chemical conditions as well as exemplary substrate specificity. Initially produced from the human pancreases, filamentous fungi are game changers in production of phospholipases and lipases. Fungi have an upper hand than other microbial source because of extracellular production, bringing down the down streaming cost significantly. The *Penicillium, Rhizopus, Mucor, Aspergillus, Beauveria, Ophiostoma, Geotrichum, Humicola, Fusarium, Rhizomucor Alternaria, Eurotrium* and *Acremonium* are major filamentous fungi for the production of lipases. While for commercial purposes *A. niger, M. miehei, H. lanuginosa, R. japonicus, C. rugosa R. arrhizus, R. oryzae* and *R. niveus*, *R. delemar* are widely used for the production of microbial lipases. Fungi prefer the lipase production through batch fermentation (Yao *et al.* 2021) and are active in solid state fermentation rather than submerged fermentation (Ali *et al.* 2023).

Yeast lipases: Yeast is a common name in food and other industries for centuries. Yeast too, like filamentous fungi, excrete yeast lipases in extracellular environment and is easy to grow and handle. Almost 50% of yeast lipases are produced as form of isozymes. Yeast for lipase production has been isolated from oil effluents, slaughterhouse refrigerators and whey cheese. *Candida rugosa* is one of the yeasts that are used commercially for lipases production; it exhibits greater activity in hydrolysis as well was synthesis (Ali *et al.* 2023). It is also termed as GRAS, which means they are "generally recognized as safe" and used in food industry (Yao *et al.* 2021). *Meyerozyma guilliermondii* too has been studied for the lipases production and yield good results. One of the most habitually used enzyme and most patented lipases is *C. antarctica* lipase B (CALB) (Yao *et al.* 2021). Moreover, due to use of yeast like *Saccharomyces cerevisiae* and *Pichia pastoris* as expression systems for production of recombinant proteins, researchers see a prospect in recombinant lipases production in yeast (Ali *et al.* 2023).

Bacterial lipases: These were initially detected in 1901 (Yao *et al.* 2021). Bacterial lipases are known for their multipurpose uses and production in bacteria flourishing in diverse environment like soil, industrial effluents, sediments, hydrothermal vents and seawater. Various genera such as *Pseudomonas Burkholderia*, *Bacillus, Arthrobacter*, *Archomobacter* and *Streptomyces* are known for the production of enzyme lipases. Bacterial lipases have applications in detergent industry, agriculture industry, biofilm degradation, biodiesel production but rarely in food industry. *P. mendocina, P. glumae, P. fluorescens, P. salcaligens* (Tembhurkar *et al.* 2012), *Burkholderia cepacia* and *Ba. thermoscatenulatus* are commonly used in industries. Recently a new lipolytic bacteria named *Staphylococcus caprae* (NCU S6) has been identified by Zhao *et al.* (2021), which has unique characteristic for the novel lipase production. However, the bacterial lipases are found nonspecific and constitutive in term of substrate specificity (Yao *et al*. 2021). Bashir *et al*. (2024) has also isolated the thermophilic bacteria and examined its potential for lipid production.

Microbial lipases from various microorganisms as shown in (Table 1) are preferred over plant or animal lipases because of their continuous and convenient bulk production and purification. Moreover, they have high stability, activity, affordability and can be easily modified molecularly (Ali *et al*. 2023).

Screening method for lipase producing microbes

Qualitative screening for lipase production: Microorganisms screening for lipase production depend on

Table 1: Lipase producing microorganisms

Sources of lipases	Microorganisms	References
Lipase producing fungi	*Penicillium, Rhizopus, Mucor, Aspergillus, Beauveria, Ophiostoma, Geotrichum, Humicola, Fusarium, Rhizomucor Alternaria, Eurotrium* and *Acremonium.*	(Yao *et al*. 2021)
Lipase producing yeast	*Candida antarctica, Candida rugosa* and *Meyerozyma guilliermondii.*	(Yao *et al*. 2021)
Lipase producing bacteria	*Pseudomonas mendocina, Pseudomonas glumae, Pseudomonas fluorescens, Pseudomona salcaligens, Burkholderia cepacia* and *Bacillus thermoscatenulatus.*	(Bharathi and Rajalakshmi 2019; Bharathi *et al*. 2019)

appearance of substrate on the agar plate, which is emulsified giving the appearance of clear halos, which indicate the lipase production. *Acinetobacter haemolyticus* NS02- 30 and *P. fluorescens* RB02-3 have been screened on tributyrin agar for lipolytic activity. Similarly, *Ba. aryabhattai* SE3-PB has been detected as calcium salts precipitates visible around Tween-20 agar wells due to fatty acid formation from lipid hydrolysis. Dyes can also screen microorganisms such as pH indicator phenol red, Victoria Blue B, Spirit blue and Nile blue sulfate are used for estimating lipolytic activity. The release of fatty acids results in the drop of pH which changes the color of the pH indicators dye. Some dyes such as phenol red dye used in Phenol red agar have been used for bacillus strain screening and Victoria Blue dye in triolein agar plate for *Geobacillus zalihae* spp. screening. Fluorescent dye such as Rhodomine B for lipolytic organisms and *Ba. thermoleovorans* CCR11 detection using emulsified olive oil as a substrate in plate assay. These methods are fast but are susceptible to false results (Yao *et al.* 2021).

Quantitative screening for lipase production: Lipase activity is measured quantitatively on a continuously stirred triacylglyceride emulsion by neutralization of free fatty acids released when NaOH is added until a constant end point value is achieved. Olive oil substrate is also common for titrimetric analysis. *P. monteilli* 2403- KY120354 showed lipolytic activity in the presence of olive oil substrate in a reaction mixture with incubation for 60 min at 37°C (Rasmey *et al.* 2017).The enzymatic action was inhibited and free fatty acids were measured using titrimetric analysis using NaOH (Adetunji and Olaniran 2021).

Production of microbial lipases

Under optimal conditions of fermentation, lipolytic microorganisms excrete the lipase enzyme after utilizing all media components. Lipases are mainly extracellular enzymes secreted in medium. The enzymes are secreted in the stationary phase of growth or tropophase as secondary metabolites in the presence of substrate acting as inducers. The syntheses of these metabolites vary according to the type of microbial strain, media components, and the fermentation condition and type of fermentation technologies employed. The production of Microbial lipases can take from some hours to many days. The fermentation technology used for production can be either solid state fermentation in a batch or continuous system or a submerge fermentation. In submerge fermentation, microorganisms are pre grown in nutrient broth as a suspension which render effective control on the process and enhanced yield of enzyme. Moreover, submerge fermentation helps in increased homogeneity as well as easier purification and product recover from the medium. In addition, it also eliminates the undesirable metabolites. For production of industrial biocatalysts, the submerged fermentation is a technology of choice for almost 99% cases (Adetunji and Olaniran 2021). The factors which can affect the production of lipases from microorganisms are:

Carbon sources: Carbon sources are the chief parameter of stimulating microbial growth for the production of lipases. Among carbon sources, carbon source of lipids origin is vital for production of lipase since the lipase production is inducible in nature. Thus, lipids such as oils or otherinducers like tweens, triglycerols, fatty acids, hydrolysable esters, glycerols or bile salts *etc*. For example, the lipase production is stimulated by the surfactant tween-80 which improves medium uptake and release of lipases enzyme. Similarly, various oils in low concentration (1%-5%,v/v) such as, coconut, castor, olive, sunflower avocado and soybean oils acts as inducers for the production of lipases (Martínez *et al*. 2019). High concentration of oil decrease oxygen transfer resulting in poor microbial growth and poor lipases production. Other sources of carbon such as polysaccharides, sugar alcohol, casamino acids, whey and other complexes too influence the production of lipase. For example, *Streptomyces griseochromogenes* produce lipase when mannitol is added in the medium. For some microorganisms, oil and carbohydrates mixtures can induce lipase production. Beef tallow, agro-industrial wastes, whey and wool scour effluents are some unconventional carbon sources incorporated in medium for the production of lipases (Melani *et al.* 2020).

Nitrogen sources: Besides carbon source, also important are the nitrogenous sources for fermentation process of the production of lipases (Bharathi and Rajalakshmi 2019). The presence of nitrogen sources including inorganic or organic nitrogen sources affects the amount of yield of lipases production in microorganisms. Yeast extract or peptone or their combinations are organic source of nitrogen, which improves the production of lipase significantly in most microbial strains. Corn steep liquor and soybeans meal produce optimum yield of lipase production. While, ammonium molybdate, diamine hydrogen phosphate, ammonium chloride are inorganic nitrogen sources effective for maximum lipase yield (Adetunji and Olaniran 2021). On the other hand, microorganisms prefer organic nitrogen

because they contain some vitamins, minerals and other growth factors. Amino acids are also included in some cases to improve the production of lipase. This can be cemented by the inclusion of poly alanine in the fermentation medium of *Streptomyces griseochromogenes* as a preferred nitrogen source. However, due to nitrogen metabolite repression a higher nitrogen concentration can inhibit the lipase production in microorganism (El-Batal *et al*. 2016). Fungi need more nitrogen than other microbial lipases production. Peptone and ammonium sulfate are effective for lipase production in *Rhizopus* (Riyadi *et al.* 2017).

Physicochemical parameters

Besides nutritious components, the high lipase production greatly depends on the physiochemical parameters like the pH of the medium, the temperature presence of metal ions and incubation period. The medium initial pH is crucial. Usually, the bacteria produce lipases at the neutral to basic pH while the fungi and yeast produce lipases at acidic to nearly neutral. Like pH, microorganisms have specific optimum temperatures for improving yield. This temperature coincides with the optimum temperature for growth of microorganisms. For example, *Aspergillus niger* produce lipases at 24°C, *Bacillus* sp. RSJ1 at 50°C *Ba. aryabhattai* at 40°C and *Bacillus* sp. produces lipase at SP5 at 37°C. Similarly, different microorganisms produce lipases at different incubation periods such as the incubation period for Acinetobacter *baylyi* G40 is 12 h while *Arthrobacter* sp. BGCC-490 and *Pseudomonas* sp. LSK 25 has optimum incubation period of 48 and 36 h. Metal ions can both improve as well inhibit lipase production. Divalent ions such as Ca^{2+}, Mg^{2+} and Fe^{2+} increase lipase production. Agitation speed is also important because it repels oil micelle, permits contact with microorganisms and increases transfer rate of oxygen (Adetunji and Olaniran 2021).

Purification Strategies for Microbial Lipases

Greater care must be taken to choose the purification techniques for the lipases to retain its activity. There are various classical and modern purification techniques available to purify the enzymes as per this objective. The improvement in purification technology has provided greater choice for the selection of purification techniques for microbial lipases.

Classical purification techniques

Microbial lipases are extracellular enzymes so after initial separation of enzyme from cells by filtration or centrifugation, precipitation and chromatography are attempted for purification. Usually all purification schemes (almost 80%) employed a precipitation step. The precipitation is carried out through ammonium sulfate, acetone, alcohol and HCl. Ammonium sulfate is two times more commonly used for precipitation as compared to acetone or alcohol, following precipitation and one or more chromatography techniques can be employed. A study by Adham and Ahmed (2009) have precipitated a crude enzyme which is partially purifies and treated it with saturation with 30%, 50%, 60%, 70% and 80% ammonia sulfate and then dialyzed it with H_2O. The study concluded that the precipitation with the ammonium sulfate saturation 70-80% show maximum lipase activity of 48% (Saxena *et al.* 2003).

Similarly, Bharathi *et al.* (2019) use cell-free supernatant in dialysis and precipitation process, with ammonium sulfate saturations ranging up to 80% (w/v). The best saturation was estimated to be 40–60% (w/v) of ammonium sulfate. The precipitates were further purified by dialysis membrane. It was concluded that when the saturation of ammonium sulfate increases, it increases the lipase activity too. However, a previous study suggested a higher saturation more than 80% can halt activity. But, precipitation procedures result in higher yields as compared to other techniques that are used (Saxena *et al.* 2003). Iftikhar *et al.* (2011) reported three steps lipase purification with precipitation followed by two steps of ion exchange chromatography. A single purification step is insufficient. The desired method to choose for purification depends on the lipase production, purification scheme and potential use. In routine classical purification the ion exchangers such as DEAE and CM are followed by size exclusion chromatography (Saxena *et al.* 2003). Classical techniques are multistep, laborious, non-specific and the purity level achieved is adequate.

Modern technologies of purification

Modern purification includes high yield purification techniques with less process time than classical purification techniques. This technique is significant in present industrial scenarios where there is a huge demand of speedy, high yield and economical purification techniques. The modern purification techniques are specific, large scale, and highly purified enzymes. These are some modern purification techniques and their commercial values (Bharathi and Rajalakshmi 2019).

Immunopurification: The purification through utilizing antigen - antibody procedure utilizing the affinity interaction of macromolecules through affinity chromatography is called immunopurification or immunoaffinity chromatography. It is a highly specific technique used for proteins purification. In immunopurification, the monoclonal and polyclonal antibodies form immune complexes with the target proteins and separate them. After forming an immune complex lipase activity has been estimated to be 92% for VNH9 and in case of BF11 it was found to be 99%. Thus, the use of monoclonal antibodies for lipase purification holds great potential (Hecht *et al.* 2022; Zhang *et al*. 2022).

Reverse Micellar Systems (RMS): A reverse micelle has

two parts, an aqueous microdomain facing the surfactants surrounding core and interacting through hydrophobic chains with the non-polar organic solvent. Extraction may be by reversed micelle or forward micelles. In the forward extraction, the lipase moves from aqueous medium to organic phase in reversed micelles in encapsulated form deriving protection from the organic solvent. In reverse extraction, the lipase doesn't encapsulate in reversed micelle for long time and simply transported to aqueous medium (Goswami 2022). Surfactants are currently used in RMS routinely as they have been found to reduce the surface tension and enhance the solubility of organic compounds. However, the complete understanding as to how the enzyme and surfactants interact are still gapped. It has been demonstrated that the active conformation of some lipases is stabilized due to presence of nonionic surfactants which mimic the substrate of enzyme lipases. Although such interactions of surfactants has not been studied. Shehata *et al*. (2023) reported that ionic surfactant-captured lipase structures do not exist. The natural substrate of lipases, lipids hold strong resemblance to the structure of the surfactant. This is the reason that during the crystallization studies, surfactants capture lipases open conformation (Carvalho and Cabral 2000). Although by combining the classical purification with reverse micelle system for *Pseudomonas* origin lipase purification and declared that reverse micelles approach led to 80% recovery in 30–40 h (Gaikaiwari *et al*. 2012). For bacterial sources, the reverse micelles are the most economical and fastest technique for lipase purification. Similarly, Fernandes *et al*. (2004) reported that lipase from *Thermomyces lanuginosus* and *Thermophilic* produce lipase yield of 200 U/mg.

Aqueous two-phase system: Aqueous two-phase system is a highly selective, single step technique with easy scale up. This technique uses two distinct liquids to liquid extraction medium. The separation of biomolecules depends on the incompatibility of the two liquids and by exploiting this property the ATPS separate and purify cellular components and biomolecules. This technique uses salt and polymer for separation. One such ATPS used by Souza *et al*. (2015) consist of tetrahydrofuran and KH_2PO_4 buffer for purification of lipases extracted from *A. niger*, *C. antarctica* and *Bu. cepacia*. This technique works in a continuous operation mode, has low toxicity from forming no phase chemicals, needs little energy, is economical consumption, high bio compatibility, environmentally friendly and is a quick separation process. Through these unique characteristics the ATPS demonstrated its utility to meet the industrial standards as a beneficial alternative.

Aqueous two-phase flotation: Another novel technique proposed by Bi *et al*. (2009), initially for separation and concentration of antibiotic Penicillin G. This Aqueous Two-Phase Flotation is a combination of solvent sublation and ATPS. It is simply the separation of biomolecules by solvent sublation in an aqueous two-phase system ATPS. This technique is more effective concentration and separation if biomolecules are in aqueous phase. It requires little organic solvents consumption. In technique gaseous nitrogen is forced in enzyme-rich solution mainly fermentation broth, the enzyme along with nitrogen is then dissolve in top phase which is polymer. It is environmentally friendly and inexpensive technique for purification of lipases enzyme in a fermentation broth. Further optimization studies are required to determine the quantity, pressure, froth and bubble size of nitrogen (Ali *et al*. 2023).

Immobilization of lipases

To improve the activity, reusability and control over the enzymatic reaction of lipase, the enzyme is immobilized with solid support. The immobilization of expensive lipases makes it easy to control reaction parameters such as flow rates as well as substrate convenience. To make good support for immobilized enzymes, the support must have thermal and mechanical stability, low cost, reusability, insolubility. Immobilization improves the overall proficiency of the lipase. The lipase can be immobilized by two ways either by physical methods in which the interaction the enzyme will have with the support are weaker such as hydrogen bonding, and vander-waals interaction or it can be immobilized by chemicals methods through irreversible covalent bonding (Prasad 2011).

Physical methods: The physical methods of immobilization include adsorption, encapsulation and confinement. The adsorption approach of physical immobilization the lipase is adsorbed to the surface of material such glass pore, charged resins, alumina, silica gel, natural material such as agarose and cellulose through the hydrophobic interaction, vander-waals and hydrophobic interaction. This is a low-cost procedure conducted in modest conditions. In encapsulation the enzyme is enclosed with synthetic or polymeric substances (Chandra *et al*. 2020). However, this method may deactivate the enzyme. Finally, confinement of the enzyme is the imprisonment in the immobilized support. The enzyme is immobilized with the reactive subunit of the matrix. This technique is particularly useful for enzyme with application in biosensor where there is little conformational changes of enzyme structure (Suckling and Suckling 1974).

Chemical methods: The lipase can be immobilized by the covalent bonding between the side chain amino group and the functional groups of support such as imidazole, carboxylic and phenolic groups. This immobilization provides stability to enzyme and prevention from denaturation agents like, pH, heat and organic solvent. Cross-linking is another chemical immobilization technique. It did not involve support. The crosslinking agent forms intermolecular or intermolecular linkage with the surface amino group side chains to prevent exposure to environment. This method results in a more stable, highly catalyzed, low-cost lipase for industrial use. the various adaptation of this procedure are Cross-Linked Enzyme

Crystals (CLEC) done for crystallized enzymes, Cross-linked Enzyme Aggregates (CLEA) done for precipitated enzymes, and Cross-Linked Spray drying Enzyme (CSDE) are used for the spray dried enzymes (Wiseman 1975; Chandra *et al.* 2020).

Applications

Role in textile industry: Lipases are used to decrease the raw material and increase the performance of fabric. Wool is treated to hydrolyze the fatty acids with anhydrous lipases on the surface of wool fiber. The lipase effect on dewaxing and degumming has been proved to have been associated with the better quality of fabric such as microstructure, wettability, dyeing, loss of weight and shine. In denim desizing, lipase is used to treat cracks and streaks. Lipase can also generate environmental friendly waste as they desize cotton fiber and degrade the starch to water soluble compounds (Gurung *et al*. 2013).

Use in detergent industry: Hydrolytic lipases are of great commercial value for their use in detergents employed for household dishwashers and laundry businesses. The use of lipases makes the detergent more environmentally friendly, overall reducing environmental impact of enzyme. The detergent with lipases and other enzymes need lower wash temperature, reduced chemicals are biodegradable, pose no threat to aquatic environment when released to water bodies as well as they didn't disturb the waste treatment process and leave no chemical traces behind (Gurung *et al*. 2013).

Use in food industry: In the food industry, the lipases break the lipids molecules to synthesize short-chain fatty acids esters and alcohols which modify the food aroma and taste by imparting flavor and fragrance. Before, lipases from diverse origin has been exploited for improving aroma, refine rice flavor, to speed up fermentation process as well as improving taste of wine and modify the soybean milk (Priyanka *et al.* 2019). The use of lipases is significant for manufacturing sausage in fermentative steps. During ripening, it also determines the number of long-chain fatty acid liberated in the process. The addition of lipases remove the fat and process meat and fish through biolipolysis (Panesar *et al*. 2010; Sandoval and Herrera-López 2018). Lipolysis also play significant role in diary industry and making of products such as swiss cheese (Mir and Selamoglu 2020).

Role in medical and diagnosis: In diagnostic field, lipases are also considered breakthrough in detection of infection or diseases as enzymes acting as target sites for diagnosis. If the increase level of lipases is detected, it indicates a disease or an infection. Such detection can serve as a diagnostic tool for medical technologists. Lipases are used to measure glycerol liberated from serum triglycerides and thus determine their concentration by colorimetric reactions. Detection of lipases presence in blood can also determine diseases such as pancreatic injury and Acute pancreatitis (Chandra *et al.* 2020). Another test "feline pancreatic lipase immunoreactivity fPLI" was developed and it was found to be accurate as compared to other diagnostic tools. Lipases play an innovative role in infection control and treatment in humans. Lipases may have antibacterial properties such as the lipase enzyme from *Galleria mellonella* was found to be bactericidal for *Mycobacterium tuberculosis* (MBT) H37Rv. The research conducted was for global hunt of promising new drugs. This is also important as to the face of the arising antibiotic resistance faced by most conventional drugs. Lipases can improve digestive action as well. Lipase activates tumor necrosis factor thus they are helpful in malignant tumors treatment. Human gastric lipase (HGL) is a promising Enzyme substitution therapy too because of its stability. Previously, dyspepsias cutaneous effects of digestive allergies, gastrointestinal disturbances and many other diseases have been treated by lipases. Lovastatin, a drug synthesized from *C. rugosa* lipase is used to decrease cholesterol level of serum (Chandra *et al.* 2020). *S. marcescens* lipase hydrolyze 3-phenylglycidic acid ester asymmetrically act as an intermediate for diltiazem hydrochloride synthesis. Diltiazem hydrochloride is a common coronary vasodilator (Gurung *et al.* 2013; Joshi and Kuila 2018).

Role in cosmetics: Lipases are active ingredients of hair, nail and face cleaning. They are both used as active ingredient or biocatalyst of chemicals used in cosmetic (Yao *et al.* 2021). Retinoids, which is vitamin A and its derivatives are used as skin care products commercially in cosmetics and pharmaceuticals industry. Water-soluble retinols derivatives are prepared by immobilized lipases. Also, lipases are an important ingredient of anti-obese cremes or orally administered anti-obese medicines. Lipase are also used in hair waving (Joshi and Kuila 2018).

Use as biosensor: Lipases role as biosensor is garnering interest because of their broader substrate specificity. The lipases acting as enzymatic biosensors are used in analytical methods in field such as the food industry, diagnostic tools, oleochemicals, biodegradable polymers and environmental science. For example, lipases act as biosensor to detect high cholesterol and triglycerides levels in blood in diagnostic labs. There are various lipases which can inhibit the activity of lipases such as many studies have reported different inhibitors for different origin lipases. Such as lipase originating from *Ba. cereus* is inhibited by cetyltrimethyl ammonium bromide, lipase from *Mycobacterium tuberculosis* is inhibited by cobalt-II and lipase from *Yarrowia lipolytica* is inhibited by Cadmium-II. Furthermore, lipase can be sensor in fields such as optical biosensors electrochemical sensors and manufacturing of different bioassays and biosensors (Sandoval and Herrera-Lopez 2018).

Use in biodegradation: Lipids (oil, triglycerides, wax and cholesterols) are the principal substrate for the lipase enzymes. They are used to treat the soil, coastal and water environmental spills by degrading its substrates (Pacheco *et al.* 2015; Alabdalall *et al.* 2021). Lipase is used for eco

restoration. It has been found that the soil microbial flora lipase activity is critical in bioremediation of freshly contaminated soils (Blandon *et al.* 2022). *Ba. licheniformis, Ba. subtilis, Serratia marcescens, Ba. amyloliquefaciens, S. aureus* and *P. aeruginosa*, originated lipases were reported to degrade diary, domestic, palm oil plant, soap industry and slaughterhouses waste waters. Similarly, lipase from *P. aeruginosa* are recommended for degradation of castor oil (Jacob *et al.* 2022). Abubakar *et al.* (2024) has isolated the *Ba. subtillis* and *P. aeruginosa* from contaminated hydrocarbons and examined their degrading activity.

Role in paper industry: Lipases are used to hydrolyze the pitch, an insoluble component of wood. Pitch is made up of triglycerides and waxes. Insoluble Pitch interrupts the smooth paper making process and microbial lipase can hydrolyzes the pitch without any adverse effect on the either paper quality or environment. Pitch control method by the application of microbial lipase was first developed by Shehata *et al.* (2023) at Nippon Paper Industries in Japan by lipase - catalyzed triglycerides hydrolysis. After the bright outcome of the process in reducing pitch significantly, the protocol was adopted in the paper making industry. Microbial lipase from *C. rugosa* can hydrolyze triglycerides and waxes up to 90% and *C. cylindrical* was successful in hydrolyzing esterified lipids 30% in fresh birch pulp and showed promising results for hydrolysis of triglycerides. The reaction considerations for these processes include concentration of enzyme, rate of continuous mixing, temperature and reaction duration (Farrell *et al.* 2006; Jacob *et al.* 2022).

Use in biodiesel production: Lipase is known to have role in production of biodiesel from oil and fats for last three decades. However, lipase practical application in biodiesel production is still at early stages. Lipase can be used to produce the biodiesel through esterification and transesterification of oils and fats. In industrial scale, biodiesel production is still chemically catalyzed. The lipases role in biodiesel production is also necessary to cement its role as net zero fuel (Pacheco *et al.* 2015). Presently, free lipases are used by researchers to produce biodiesel while many other studies have suggested immobilized lipases. Microbial lipases are used frequently for the production of biodiesel. For diesel production, microbial lipases are exploited on account of diverse origin, formulation, increased activity, thermal tolerance, short reaction time, immobilized enzymes reusability and lower production inhibition. Usually, lipases from microbes such as *C. anuginose*, *Ba. aureus* and *Rhizopus arrhizus* are used to produce lipases (Joshi and Kuila 2018). In general, the microbial lipases applications are very diverse. Almost all industries have successful application of microbial lipases (Fig. 2).

Fig. 2: Industrial applications of microbial enzymes (Yao *et al.* 2021)

Conclusion

The microbial lipases are the most common lipases and are preferred over other sources because of their cost effectiveness, availability, high yield, easy production and purification. Lipases can degrade any ester bond that is present in plastics, insecticide, pesticides, domestic and industrial waste etc. Oil spills are the major issues in countries which import or export oils through sea route. The breaking of these oil components can prevent marine diversity against extinction. Furthermore, lipases in medical, diagnostic field and as biosensor hold great importance for researchers. In the near future the role of lipases as the medical field and in bioremediation is promising and bright.

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Author Contributions

HI collected data and conducted write up of this review articles. SA planned this manuscript, AA helped with the editing. AW and SWK proofread the manuscript.

Conflict of Interest

There is no conflict of interest associated with this article.

Data Availability

All the data would be made available at the fair request to the corresponding author.

Ethics approval

Not applicable

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