Microbial lipase and its industrial applications

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Abstract- Lipase enzymes are used in a variety of industrial processes, including biodiesel, oils, food and beverages, leather, textiles, detergents, pharmaceuticals, cosmetics, paper, cleaning, chemicals, and pharmaceuticals. Lipase-producing bacteria have been isolated from oil-contaminated soil. Screening for enzyme-producing bacteria. Tributylene acid is commonly used to control lipase production by microorganisms. A distinct halo zone is formed around the colonies by using olive oil as a substrate. Enzyme activity is measured from isolated colonies of inoculated cultures and after 24 hours at 37°C. on a rotary shaker. Cultures were centrifuged at 10,000 rpm for 10 minutes and the cell-free supernatant was used as the enzyme source. Lipase activity in the supernatant was measured by titration. Lipase enzymes are used in the textile, detergent, and dairy industries.

Keywords: Lipase, Extraction, Lipid hydrolyses test, Tributyrin, Extracellular enzyme, Optimization.

I.INTRODUCTION

Lipases, also known as triacylglycerol ester hydrolases (EC3.1.1.3), are enzymes that naturally cleave triglyceride ester bonds to produce glycerol and free fatty acids [1,2]. Lipids make up the majority of the Earth's biomass, and lipolytic enzymes play an important role in the turnover of these water-insoluble compounds [3]. Cells of individual species use lipolytic enzymes to break down and transport lipids, moving lipids between cells. It becomes a lipid from one organism to another [4]. The lipase family is one important category of biocatalysts used in biotechnology applications [5]. Several plant, animal, bacterial, fungal, and yeast species lipases have been isolated [6]. Microbial lipases are used by many companies such as dairy, food, detergent, textile, pharmaceutical, cosmetics, and biodiesel [7]. The high commercial potential of microbial degradative enzymes has stimulated research in this area [8]. Microbial enzymes are not only safer and easier to produce, but they are also more stable than their respective plant and animal enzymes [9]. Many environments have been shown to contain lipase-producing microorganisms, including industrial waste, vegetable oil processing plants, dairy farms, and oil-contaminated soils. Microorganisms such as fungi, yeast, and bacteria are known to have hidden lipases [10]. Lipases are employed in manufacturing biodegradable polymers or compounds, the detergent industry as an addition in washing powder, the textile industry to promote fabric absorbency, and other industries in several trans-esterification processes [11]. Most bacterial lipases are extracellular, and dietary and physicochemical variables significantly impact them [12]. The more affordable and stable bacterial lipases out of all of these [13]. Activities that significantly led to environmental deterioration were dramatically expanded by the industrial revolution of the 19th century [14]. Enzyme catalysis is one of the sustainable production methods that manufacturing businesses have developed in response to the current status of the environment and the need to preserve it [15]. Enzyme catalysis works under relatively mild temperature, pH, and pressure conditions, which consumes less energy and minimizes the production of unwanted by-products [14]. The most suitable method for producing microbial enzymes in the field of biotechnology is submerged fermentation, which is also a promising technology in developing countries [16]. Applications of lipases are selected based on their substrate specificity, regio- and stereospecificity, and temperature and pH in various industries [17,18]. Oil demand is increasing worldwide, leading to increased waste production and waste-related environmental damage. One of the biggest problems in the world today is oil. An industrialized world that damages the environment [19]. Microbial lipases also have short generation times and bacterial cells are easy to genetically manipulate [20]. Lipases are naturally produced by the stomach and pancreas of animal species for the digestion of fatty acids and lipids [21]. Microbial enzymes are used in various industries such as B. Food production, fermentation, agriculture, pharmacy, and chemical processing. More than 500 commodities are manufactured using microbial enzymes, which have long been used by industrial product manufacturers as key catalysts for converting raw materials into finished products [22]. Their chemical reactions form six types of enzymes: 1. oxidoreductase 2. transferase 3. Hydrolase 4. lyase 5. isomerase 6. ligase [23].

II.HISTORY

Wilhelm Kühne was the first to use the term 'enzyme', and a few years later Emil Fisher proposed a 'lock-and-key model' to visualize the interactions between substrates and enzymes [24]. Biocatalysts are enzymes. The word "enzyme" comes from the Greek word "enzumé", which means "in yeast". Kuhne first used this expression in 1877[25]. Clade Bernad identified lipase in pancreatic juice in 1856 as an enzyme that breaks down insoluble oil droplets and converts them into soluble molecules [26]. Various types of fungi, yeast, bacteria, plants, and animals have been reported to be sources of lipase. However, everyone is talking about microbial lipases because of their potential for many industrial applications, simple culture operations, and simple scaling during production [14].

III.SOURCE OF LIPASES

i.Plant lipases

Lipase is a common esterase enzyme produced by microorganisms, plants, and animal cells. Plant lipases have been found in leaves, oils, stems, latex, seeds, seeds, and grains of oleaginous plants. Various applications of plant lipases are well-established reactions involving biotransformation and vegetable oils, although further research is needed for their application in the production of concentrated FAs from plants [27]. The most effective method of producing biocatalysts from plants depends on the plant components and the desired degree of purification of the plant biomass [28]. These enzymes are particularly attractive due to their ease of purification, low cost, and versatility [29]. Lipases from various sources of seed plants such as dormant castor bean (Ricinus communis), sunflower (Helianthus annuus), maize (Zea mays), and passion fruit (Passiflora edulis) undergo oil hydrolysis to produce concentrated FAs. has been evaluated [27]. In addition, a crude extract from germinated seeds of Jatropha curcas L. also showed good ethanololytic and hydrolytic activity [30]. However, due to the lack of research on the optimal production of plant lipases, the intrinsic substrate specificity of plant lipases for industrial applications has not yet been fully determined [31]. A novel plant enzyme extracted from physical nut seeds and capable of hydrolyzing various biodiesel bases were characterized. The detected lipase activity was 111 ± 19 U/g for tributyrin and 106 ± 106 for tricaprylin and 9 U/g and $96 \pm$ U/g for olive oil. These results indicate that this plant enzyme showed no selectivity for the triglyceride chain lengths tested [29]. Direct application of lipase crude extract from seed oil offers advantages in the oleochemical industry as all purification and immobilization steps are avoided, reducing production costs. Biocatalysts can be easily recovered because the enzymes are naturally immobilized on the solid material from the seeds [32].

ii.Animal lipases

Animal lipases used in modern cycles include pancreatic and forestomach lipases. In any case, only those derived from the porcine pancreas are used in modern applications. These compounds are used in the production of lysophosphatidylcholine or lysolecithin food-grade emulsifiers and antifungal agents [33]. Microorganisms, yeasts, and filamentous parasites are currently the main sources of industrially viable compounds. Regardless, porcine and the human pancreas were the first sources of lipases and phospholipases used in food processing. This shift is because both lipases and phospholipases from normal sources consistently fail to meet the requirements of modern biocatalysts in their formulation of motion, strength, and character [34]. Animal lipases are obtained from children, calves, and lambs [35]. The majority of somatic cells hydrolyze her TAGs with lipases, providing FAs to meet energy needs through similar pathways [36]. All extrahepatic tissues have lipoprotein lipases that hydrolyze circulating TAGs to free FA and glycerol. Cardiac and mammary gland activity is highest [34]. Moreover, its tissue-specific regulation controls the flow of TAGs circulating in the body [37]. The highest esterification activity (1403 U/g) was reached after 180 min of immobilization when the mass ratio of the enzyme to the carrier was 2:1 [34].

iii.Bacterial lipases

Most microbial lipases are extracellular and the type of medium has a great influence on how they are produced. The production of lipase can use agricultural industrial waste as a starting material at a low cost, solving the waste problem of such objects [38]. Microbial enzymes are often more useful than plant- or animal-derived enzymes because of the broad spectrum of accessible catalytic activity, the high yields that can be achieved, and the ease of use. Genetic engineering, constant supply due to lack of seasonal variation, and rapid microbial growth on inexpensive surface media [9]. Although lipases are primarily lipolytic enzymes, they also exhibit osteolytic activity and exhibit broad substrate, chemical, regio- and enantioselective, and highly specific catalysis [39]. Extracellular bacterial lipases are of great commercial importance due to the ease of large-scale synthesis. Although there are many sources of lipase-producing bacteria, only a few have been used commercially as wild or recombinant strains [40]. Of these, the most important are Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Burkholderia, Chromobacterium, and Pseudomonas. Among them, the bacterial Pseudomonas lipase is widely used for many biotechnological purposes [40]. When Staphylococcus pasteurii was isolated from oil-contaminated areas and cultured on coconut oil mill residue, it produced large amounts of extracellular synthesis of highly active lipase. Distillation waste can be a good substrate for bacteria to grow. This species has been shown to produce a lipase that is thermostable and resistant to organic solvents and can be treated with 0.5% (v/v) inoculum, agitation, and 2% (v/v) castor oil (inducer) reaches 14.5 U/mL when spun at 150 rpm [34].

iv.Fungal lipases

fungi capable of producing lipases can propagate in a variety of habitats, including seeds, used vegetable oils, oil-contaminated soils, and spoiled meat and dairy products [41]. The most frequently cited genera for lipase production are Fusarium, Aspergillus, Rhizopus, Penicillium, and Mucor [42]. Furthermore, filamentous fungi are considered to be the best source of extracellular lipase for large-scale commercial production of all organisms used as a source of lipase [34]. Moreover, the value of lipases for organic synthesis is increasing due to rapid advances in molecular biology techniques and the availability of more reliable high-throughput screening methods [41]. Second-generation biocatalysts are now made by tailoring wild-type enzymes for specific applications [41]. New strains of lipolytic bacteria are used [41]. For each application, several methods have been developed to increase the conversion of highly specific enzymes, increasing the potential for lipases to be used in industry [41]. Due to their stability, selectivity, and broad substrate specificity, fungal lipases are of particular industrial interest [9]. Aspergillus terreus, Rhizopus homothallic, and Mucor pusillus are all thermophiles and are known to produce thermostable extracellular lipases. Extracellular, inducible, alkalophilic, and thermostable at high alkalinity and high-temperature lipases are unusual. Mucor sp. produces such a lipase. Currently, few reports are known about fungi that produce alkalophilic and thermostable lipases [43,44]. It is well known that fungi play an important role in bioremediation processes in various environments. Industrial and household wastes contain bacteria that break down fats and oils better. In addition to litter removal, biotransformation by fungal activity produces a variety of usable materials. This review focuses on methods to characterize fungal lipases, with emphasis on strategies to characterize fungal lipolytic enzymes from different waste sources for many different applications [45].

IV.LIPOLYTIC ACTIVITY

pure isolated bacterial cultures were tested for lipase activity using tributyrin agar [46]. Pure cultures were separately streaked onto tributyrin agar plates and incubated at 37°C. for 24 hours. After incubation, prominent hydrolysis zones of colonies around them showed the presence of lipase activity. Positive cultures showing the greatest zone of hydrolysis were selected for additional testing. Bacterial isolates were screened for lipolytic activity on Tween 80 agar. Each pure culture was separately streaked onto Tween80 agar plates and infected plates were incubated at 37°C for 48 hours. A white precipitate around the colonies after incubation indicates lipase activity. Each pure culture was separately streaked onto rhodamine-olive oil agar plates and infected plates were incubated at 37°C for 48 h. The appearance of orange fluorescent halos around the bacterial colonies during incubation, visible when exposed to UV light, indicates the production of lipase. A loop of each pure culture was streaked onto phenol red agar and incubated at 37°C for 48 hours. After incubation, the plate changed from orange to pink [14].

V.Production of Lipase Enzyme

i.Submerged Fermentation

Precultures were maintained in a medium of Luria Bertani Broth. Concentrations of inoculum, substrate, nitrogen source, inducer, and Ca2 metal ion were used to determine peak lipolytic activity after the addition of cells to the production medium. Peptone, yeast extract, and substrate are mixed at 150 rpm in phosphate buffer (0.1 M pH 7.5) at 30°C. Cultures were fermented for 84 hours in 100 mL Erlenmeyer flasks on a shaking water bath. The basis for increased lipase production is the ideal variation conditions described above [47].

ii.Solid Phase Fermentation

microbial lipases are mainly produced by submerged fermentation (SmF). This is a well-known process whose technical aspects are now well-developed. However, solid-state fermentation (SSF) has shown some advantages in enzyme production compared to SmF, even at a commercial scale [48]. Only a small percentage of free water needs to flow and uses inexpensive substrates such as agricultural waste. It serves both as a nutrient source for the fermentation process and as a support for microbial growth. Solid-state fermentation studies with various waste products and microorganisms have achieved good results at low enzyme production costs. Among these microorganisms, filamentous fungi are considered the most suitable for processes involving SSF due to their ability to grow in small amounts of free water and their efficiency in degrading some contaminants. In particular, the filamentous fungal production of lipolytic enzymes in SSF from agro-industrial waste has attracted a great deal of interest because these enzymes are mostly extracellular and can be easily extracted from the fermentation medium. Fungi of the genera Rhizopus, Mucor, Rhizomucor, Geotrichum, Penicillium, and Aspergillus have been reported to be lipase-producing organisms. Among them, the fungus Aspergillus niger is considered promising for lipase synthesis for industrial use due to its ability to grow rapidly on solid supports and synthesize large amounts of extracellular lipase [49]. Several variables influence lipase production by filamentous fungi. For example, carbon and nitrogen sources, medium composition and pH, fermentation temperature, bioreactor geometry, etc. Therefore, this study aims to evaluate the influence of medium composition on lipase production by Aspergillus niger grown in semi-subsistence farms using agro-industrial waste supplemented with various carbon sources. [50].

iii.Lipase Assay Procedure

selected bacteria were tested for extracellular lipase production using a titration method using olive oil as substrate. Olive oil (10% v/v) was emulsified with gum arabic (5% w/v) in 100 mM potassium phosphate buffer pH 7.0. 0.1 ml of extracted crude lipase was added to the emulsion and incubated at 37° C. for 15 minutes. The reaction was stopped and 1.0 mL of acetone: ethanol (1:1) solution was added to extract fatty acids. The amount of released fatty acids was estimated by titration with 0.05 M NaOH to pH 10.5 using phenolphthalein as an indicator [51].

One enzyme unit is defined as the amount of enzyme required to hydrolyze µmol fatty acids from triglycerides.

Lipase Activity = (NaOH Volume * NaOH Normality) / (Incubation Time * Enzyme Volume)

iv.Purification of Lipase

The culture was centrifuged at 8,000 rpm in a refrigerated centrifuge for 20 minutes at 4°C. After saturating the cell-free supernatant with 70% ammonium sulfate, it was centrifuged at 14,000 rpm for 20 minutes at 4°C. with continued stirring. The ammonium sulfate fraction in the dialysis bag was dialyzed with 50 mM Tris-HCl buffer (pH 8.0) at 40°C for 6 hours. After dialysis, the concentrated enzyme was applied to a Sephadex G-100 column. A flow rate of 1 milliliter per minute was used to elute the enzyme from the column. Protein concentrations were measured spectrophotometrically at 280 nm after collecting the enzyme fractions. A lipase experiment was performed using the fraction with the highest protein content [52].

VI.APPLICATIONS OF LIPASES

i.Lipases in the Detergent Industry

The enzyme industry has made significant strides to market lipases as a second group of enzymes after the great commercial success of enzymatic detergent additives for detergents. have made great efforts [53]. The use of detergents is increasing because of their ability to impart softness, antistatic properties, and other water-insoluble benefits to materials [54]. Lipase enzymes are commonly used in detergent formulations to remove greasy stains such as lipsticks, edible oils, salad oils, butter, sauces containing fat as an ingredient, and soups [55]. Lipase enzymes are free of harmful residues, energy-saving, and biodegradable, by allowing additional ingredients that do not adversely affect the wastewater treatment process, are unattractive, and do not pose a risk to aquatic life.

ii.Lipases in the Food Industry

Commercial lipase-based technologies use mixed hydrolysis and synthetic methods to remove less desirable lipids. Some have been employed to convert it into a substitute for cocoa butter [57]. A variant of this method replaces palmitic acid in palm oil with stearic acid by transesterification using an immobilized lipase [58]. by enzymatic action such as B. Lipolysis, intracellular enzymes are

released after cell lysis and impart flavor. Cheese made from unpasteurized cow's milk is infected with Pseudomonas. It has a lipolytic effect, especially in the production of blue and camembert cheeses [59].

iii.Lipase in the pulp and paper industry:

Paper mills frequently have challenges caused by pitch. Pitch control technique that hydrolyses the triglycerides in wood using fungal lipase [60]. In the paper business, lipase may typically improve whiteness and intensity, minimize chemical use, lower the amount of wastewater pollution, and lower the cost of composite materials [61]. By eliminating the pitch from the resulting pulp, lipase is utilized in the production of paper. Nippon paper industries have discovered a pitch in Japan a technique to remove the majority of the wood triglycerides [62].

iv.Lipase in the textile industry:

The primary use of lipase in the textile industry is to degrease and enhanced the performance of textile raw materials [63]. The leather industry uses lipase to extract the fat and collagen fibers from the fur, making the fur products softer and more flexible [64]. They provide enzymes for de-sizing, stone washing of denim and jeans, enzymatic wash, biopolishing of knitwear, and all other chemical processes treatment for jeans with chemicals stone cleaning and silicon application. Lipase enzymes are employed in the textile industry to remove size lubricants and to raise the amount of dying. It is a commercial product that contains lipase enzymes that are used for designing denim and other cotton fibers [65].

v.Lipase in the pharmaceutical industry:

In the medical field, lipase is used to enhance the impact of medications by delivering them to specific regions [66]. Animal and plant lipids are converted into PUFAs by microbial lipase and a range of medicines are made from their mono and diacylglycerides [67].

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