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Microbial Lipases: Versatile Enzymes Which Hold Immense Potential in Industrial Application

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Abstract: Lipases abundant in nature have a wide application in several industrial sectors. They can be isolated from a variety of sources but lipases isolated from microbes have huge demand due to the easy availability, cost efficiency, greater yield, simple genetic manipulation and higher thermostability. The lipase market is valued at US$ 690 Mn in 2021. It is set to grow at 7.8% CAGR through 2026. The global microbial lipase market size is expected to reach US$ 915.9 Bn by the end of 2029. Lipases are known to play important role in esterification, alcoholysis and hydrolysis. Extracellular enzymes produced from microbes are in great demand due to its wide application in industry related to improving the manufactured products. Lipases have become important in applied and industrial microbiology as also in enzyme engineering sector. Thermostable lipases are in demand as it can carry out chemical reactions at higher temperature without alteration of the structure and activity of the enzyme as also faster reaction and increased solubility. They act as biological catalysts meeting the requirement in several industries like dairy, food, brewery, pharmaceuticals and medicine, detergent, leather and biodiesel. This review is focused on the different application of lipases in industry, the purification methods of lipases and the methods for their assay measurement.

Keywords: Lipases, Microbes, Biocatalyst, Industrial Application, Activity

1. Introduction

Lipases also known as serine hydrolases are abundantly present in nature and belong to the triacylglycerol ester hydrolase family (EC 3.1.1.3). They can be isolated from a variety of sources: animal, vegetable and microbiological. Lipases isolated from microbes are in huge demand due to their application in several industrial sectors. Microbial lipases are found to have increased productivity, variety of catylytic activity, simpler genetic manipulation, no seasonal variations, regular available supply and higher thermostability [1, 2]. The lipase market is valued at US$ 690 Mn in 2021. It is set to grow at 7.8% CAGR through 2026. The global microbial lipase market size is expected to reach US$ 915.9 Bn by the end of 2029. The Asia-Pacific was the largest market for lipase consumption in 2014 [3, 4]. Lipases play important role in several reactions: esterification, hydrolysis and alcoholysis. Since the discovery of lipase by Claude Berbard in 1856 scientists have increased their interest to isolate microorganisms producing lipase and analyze their character due to immense importance of this extracellular enzyme in industrial field. Several microorganisms are screened on large scale due to their demand in industrial applications which include food, textile, detergent, cosmetics, pharmaceuticals and medical. Demand for lipase is further increased due to its involvement bound to production of the biodiesel and organic synthesis.

Lipases isolated from bacteria offer higher activity and more thermostability thereby increasing its demand for use in various industries. Bacteria are ubiquitous in nature and constitute a large domain of prokaryotic microorganisms well known for its positive and negative aspects. These organisms belong to different groups based on their biochemical, morphological and physiological characteristics. Microorganisms are ubiquitous for its diverse metabolic activity and adaptiveness for which most important factors are their intracellular and extracellular enzyme systems. Enzyme is the biocatalyst playing an important role in all stages of metabolism and biochemical reactions. Enzymes are involved in all essential processes of life such as DNA replication and transcription, protein synthesis, metabolism and signal transduction etc. Diverse capabilities of microorganism make them a potential creator of different product for human welfare. The ability to perform chemical transformations and/or product making, that make them increasingly used in industrial processes as a whole or with some special enzyme of them.

Microbial system produce different extracellular enzyme to digest different complex organic molecule to be used for nutritional purpose or other means. These extracellular enzymes obtained from different micro-organisms known to be superior enzymes have wide industrial aspects. Extracellular microbial enzyme is now a potential need marker for different industry due to its ability to improve the products. Now these types of enzyme are used in different industry globally. The hydrolytic enzymes with their potentiality like amylase, protease, cellulase, pectinase, xylanase, esterase, lipase etc. become an emerging field in applied, industrial microbiology as well as enzyme engineering sector.

Most of the bacterial lipases are stable up to 50°C and give up to 75% maximum activity if incubated at for 30 minute at said temperature. The demand of thermostable lipase for industry is on growing. Thermostable lipases are more important in industrial application because even at higher temperatures the structure of the enzyme remains unaltered as also reduced risk of contamination, faster reaction and increased solubility. The present review focus on the different sources of microbial lipases with a special emphasis on bacterial lipases and their role in different industrial applications.

2. Background

Enzymes are biocatalysts which may be produced extracellarly or intracellularly. They play an important role in different catalytic reactions involving several substrates at different environmental conditions like temperature, pH, substrate concentration, inhibitors, etc. Enzyme is the biocatalyst playing an important role in all stages of metabolism and biochemical reactions. Enzymes are involved in all essential processes of life such as DNA replication and transcription, protein synthesis, metabolism and signal transduction etc. Diverse capabilities of microorganism make them a potential creator of different product for human welfare. The ability to perform chemical transformations and/or product making, that make them increasingly used in industrial processes as a whole or with some special enzyme of them. Lipase was first discovered in pancreatic juice as an enzyme by Claude Bernard in 1856 which hydrolyzed unsolvable oil droplets and transformed them to soluble products [5]. Since then lipases have been isolated from several microorganisms such as *Bacillus prodigiosus*, *B. pyocyaneous* and *B. fluorescens* in 1901 to the current era from *Serratia marcescens*, *Pseudomonas aeroginosa* and *Pseudomonas fluorescens* [6].

A new lipase was isolated from a thermophilic Bacillus which is not only thermostable but also alkali tolerant [7]. Biodiesel production by transesterification of oils using lipases have also been demonstrated [8]. A thermostable lipase have been characterised from thermophilic *Geobacillus* sp [9]. The lipase producing activity at extremes of temperature, pH, pressure, salinity for extremophiles have been studied using metagenomics. [10]. An acidic lipase from *Pseudomonas gessardii* have been demonstrated using beef tallow as a substrate for fats and oil hydrolysis [11]. A thermostable lipase isolated from *Serratia marcescens* was applied in detergent formulation and biodiesel production [12].

Kinetic and thermodynamic study of alkaline lipase from halotolerant Bacillus have been reported [13]. Experiments demonstrated that stability of lipase can be enhanced by different protein engineering techniques that include activation and stabilization of lipase by grafting copolymer of hydrophobic and zwitterionic monomers onto the enzyme [14]; enhancing the thermostability of *Rhizopus chinensis* lipase by rational design and MD simulations [15] and improving the thermostability of *Rhizopus chinensis* lipase through site-directed mutagenesis based on B-Factor analysis. Response surface methodology have been used for enhanced production of a thermostable bacterial lipase [16]. Expression and characterization of a thermostable lipase from different bacterial strains have been carried out. Commercially important lipases isolated from animal pancreas was used as a digestive syrup either in crude form or in purified grade.

3. Lipases: Properties and Mechanism Characteristics

Lipase are the triacyl glycerols which catalyses the hydrolyses of fatty acids. They catalyse a broad range of substrates which include cholesterol, phospholipids, fat-soluble-vitamins, spingomyelins. Lipases isolated from microorganisms like bacteria, fungi have an immense importance in biotechnology due to their tolerance to different environmental conditions such as temperature, pH, metal ions, etc. as well as the easy bulk production on demand. The backbone of lipase is composed of glycerol and fatty acids. Microbial lipases are found to show activity for a wide range of pH. Most work efficiently at neutral pH while some are found to have optimum activity at acidic or alkaline pH. In general an optimum pH of 4-8 have been reported for most lipases isolated from bacteria. However *A. niger*, *Rhisopus sp*, *Chromobacterium viscosum* produced extracellular lipases which are active at acidic pH and *P. nitroaedeucens* produce lipases which show activity at alkaline pH of 11.0 [17]. The bivalent metal ion calcium have been reported to enhance the activity of lipases [18.19]. However metal ions like Co^{2+}, Zn^{2+}, Hg^{2+}, Sn^{2+}, Mg^{2+}, EDTA and SDS have been reported to have an inhibitory effect on the activity of lipases. Several lipases have been reported to show activity at temperature below 50°C. However thermostable microbial lipases isolated from several bacteria like *Serratia sp*, *Bacilllus stearothermophilus* and other *Bacillus sp.* show optimum activity at higher temperatures even up to 75°C-80°C. Thermostable lipases are industrially in high demand due to their stability even at high temperature. Based on the region-specificity of lipases, they are divided into two categories. In the first group of lipases only fatty acids are released from all the positions of glycerol. The second group of lipases are those from which fatty acids are released from 1 and 3 positions of acylglycerol. Organic solvents have shown to have an effect on the activity of lipases. Hence by modulation of solvent properties the enzyme specificity and thereby activity of the industrially important enzymes can be optimised.

4. Microbial Lipases: Sources and Application in Industry

4.1. Dairy Industry

Lipases have a wide application in food and dairy industry. Starting from the hydrolysis of milk fat to rendering of characteristic flavour to cheese lipases play an important role. The characteristic flavour of cheese is brought by the action of lipases on milk fat with the generation of free fatty acids [20, 21]. *A. niger*, *A. oryzae*, etc. are used for the production of microbial lipases in cheese manufacturing. Enzyme modified cheese (EMC) is manufactured by incubation of cheese at elevated temperature in presence of enzyme using lipase catalysis to generate the desired specific flavour of cheese [22]. EMC contains 10times more fat than normal cheese and is used in the making of soups, dips, sauces, etc as an important ingredient [23, 24]. To enhance the ripening of cheese and improvement of flavour in cheddar cheese, ras cheese, etc gastric lipases are used [25]. Release of free fatty acids from triglycerides enhance the specific flavour of cheese.

4.2. Food Industry

To produce lean meat and fish products lipases are extensively used for the removal of unwanted fats from meat and fish. Lipases also help to improve the quality of fermented sausages and to add flavour to fermented meat products [26]. Microbial lipases are used for the hydrolysis of fish oils and generation of unsaturated fatty acids (n-3 PUFA). Previously dry-curring was used for meat preservation. With the advancement of technology, need is felt to preserve adipose tissue lipids and muscles from intense lipolysis to obtain a palatable meat product [27, 28].

4.3. Brewery Industry

Just like adding characteristic flavour to cheese, addition of specific aroma to wine has been attributed to the contribution from several factors which also include lipases. The ethyl acetate esters have received much attention over the years for its great influence on taste and aroma of wine [29, 30]. Genetically engineered *Escherichia coli* B21 for lipase/ esterase production containing the lipase/esterase gene drastically improved the taste and odour of wine [31]. These enzymes needed for good quality wine production was produced in high yield and showed good productivity at low pH and stability in the presence of several salts and organic solvents [32, 33].

4.4. Textile Industry

Lipases find an application in removal of grease from raw textiles and improving the quality of the finished product in textile industry. The quality of wool is improved by removal of fatty acids from raw wool fibre by treatment with anhydrous alkaline lipases [34]. Dewaxing and degumming of raw silk by using proper proportions and usage of lipases and proteases helps in augmenting the quality of silk [35]. Similarly lipases along with amylases are used for the desizing procedure of cotton fabric by removal of pollutants from waste water and degradation of starch to water soluble compounds [36].

4.5. Leather Industry

Degreasing is a necessary step in leather industry. For softening of fats from sheepskins in the production of soft leather lipases and surfactants substitute for solvent [37]. However use of surfactants and organic solvents are harmful to the environment, hence lipases serve for the purpose of leather degreasing. Lipase enzymes help in the removal of grease and fats from skins and hides [38]. Acidic and alkaline stable lipases are used for the process. Production of water-resistant and low fogging-leathers in tanning industry use lipases in combination with specific proteases [39]. The proteases help in rupture of the fat cell membrane making it accessible to the lipases. Acid lipases are used for wool and fur. Use of lime and salts of sodium sulphide for removal of hair from animal skin is no longer efficient in leather engineering industry and in its place a mixture of lipases are used for the purpose [40, 41]. Application of lipases in leather manufacturing renders the end product of high quality.

4.6. Medicine and Pharmaceutical Industry

Regioselective lipases are used in pharmaceutical industry. Lipases have been reported to be used in the treatment of hair loss and diseases of the skin scalp [42]. Lipases isolated from *Bacillus* have been reported to show selectivity to the fatty acid chain of an ester. This property of *Bacillus* lipases enables the synthesis of enantiopure compounds in the pharmaceutical industry [43]. *Mycobacterium tuberculosis* lipases are used for the high specificity and sensitivity detection of tuberculosis infection [44]. Lipases act as excellent marker enzymes in the treatment of several diseases. In acute pancreatitis, high level of lipases in blood serum level serve as a marker for the detection of the disease [45, 46]. Lipases are used in anti-obese creams as also in the treatment of malignant tumours. Cholesterol level is reduced by lovastatin drug which is produced by lipase from *Candida rugosa* [47].

S. marcescens lipase is used in the manufacture of diltiazem hydrochloride widely used for coronary vasodilation [48]. Microbial lipases are used in the manufacture of several drugs for the treatment of diseases like DOPA for Parkinson's disease. Lipases find a major application in the treatment of diseases like gastrointestinal disturbances, dyspepsia, cancer treatment as also serve as a diagnostic tool in medicine [49].

4.7. Biodiesel Production

Increased environmental pollution, generation of greenhouse gases, global warming and increase in price from fossil fuels has encouraged the need for production of eco-friendly energy resources; biodiesel technology from sustainable resources. Lipases are excellent biocatalysts which cause the conversion of the enormous lipid waste into sustainable and eco-friendly resources on the basis of biotechnological application [50]. Microbial lipases are used for biodiesel production. They can be obtained in a higher yield, easy to handle under different environmental conditions and simplicity in productivity. *Aspergillus niger*, *Candida antarctica*, *Candida rugosa*, *Streptomyces sp*, *Pseudomonas florescens*, etc. are some of the microorganisms which produce lipases utilised in biodiesel production [51, 52]. Recently, *Streptomyces sp* lipase have been used in biodiesel production [53]. The cold-adaptive lipases isolated mostly from bacteria except a few from fungi are used as attractive targets for biodiesel production. Immobilised lipases from *Pseudomonas fluorescens* and *Pseudomonas cepacia* are the most dynamic biocatalysts used for biodiesel generation from waste materials [54].

5. Purification of Microbial Lipases

Due to the easy availability and productivity in high yield most lipases are isolated and purified from bacterial and fungal sources. Most microorganisms produce extracellular lipase and hence the first step in the purification process is removal of the cells and insoluble materials from the liquid culture. Purification of lipases to homogeneity is carried out by both conventional and novel methods. Lipases are excellent biocatalysts used in several industrial applications due to the cost efficacy. Among the conventional methods used for lipase purification include precipitation method by ammonium sulfate or organic solvents, chromatographic separation by gel filtration, ion-exchange and affinity chromatography methods and ultrafiltration or dialysis techniques using membrane [55, 56]. Recent novel methods of purification of lipases include recombinant technology using genetic engineering, aqueous two-phase systems, reverse micelle systems and aqueous two-phase flotation methods. Recombinant technology where the lipase gene is cloned into a host cell and expressed with a specific tag is the most widely used method for purification of lipases in industries. Purification using this novel method not only increases the purity and yield of the enzyme but also is cost effective for production.

6. Assay of Lipases

The use of water-insoluble longer acyl chain length derivatives is considered diagnostic for lipases. Out of the common methods used for lipase assay, two measure the free carboxylic acid or fatty acid liberated by the action of lipase on natural substrates either by titrimetric method or colorimetric method. The liberated free acid is detected by titration with NaOH to a thymolphthalein end point or the liberated fatty acid is detected by complexation with a colorimetric reagent of cupric acetate. Alternatively, lipase activity can be determined by using chromogenic substrate analogs like pNPP (p-nitro phenyl palmitate) using spectrophotometric method [57]. The absorbance of the product generated is measured at 405nm as the standard solution of pNP. Activity can also be determined by titrimetric method as well as by Thin Layer Chromatography (TLC).

7. Conclusion and Future Perspective

Lipases are currently an enormous attention because of their biotechnological potential. Lipases have become excellent biocatalysts in a broad range of industrial applications including pharmaceuticals, fine chemicals, paper manufacture and production of cosmetics. The growth of industrial microbial lipases in the detergent industry is the innovative key factor in replacing harsh chlorine bleach with lipase and reduced the industrial as well as sewage pollution from fresh water. The microbial lipases in the form of powder is projected to dominate the microbial lipase markets due to its stability, easy to handle, and easier for packaging and its transportation preferred by the consumers. The ester product from short chain fatty acid has application as flavoring agents in the food industry. Oil contamination in water and soil is a worldwide environmental problem, posing a huge threat to human health and natural ecosystems. The lipid hydrolysis is part of metabolic system of bacteria, the diversity of lipase producer are ubiquitous and especially found in oil contaminated soil and water such as petroleum contaminated soil, soil contaminated with crude oil, industrial waste etc.

Enzyme biotechnology coupled with genetic engineering is a new field which is in great demand. Increase in the production rate of lipase enzyme with increased potential activities will help to decrease the cost of enzyme production. Low cost of enzyme helps in industry as well is of societal benefit.

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