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Exploring Thermostable Lipases: Molecular Innovations and Expanding Industrial Horizons

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ABSTRACT

Thermostable lipases are crucial biocatalysts valued for their stability and functionality in both aqueous and non-aqueous environments, enabling efficient catalysis at elevated temperatures. These enzymes, derived from both wild-type and genetically engineered strains, exhibit unique properties that make them indispensable across diverse industrial sectors. Despite their potential, challenges remain in optimizing their production, purification, and characterization to meet specific application requirements ranging from highly purified pharmaceutical formulations to less refined industrial uses. This review consolidates recent advances in the isolation, engineering, and detailed characterization of thermostable lipases, highlighting their substrate specificity, catalytic efficiency, enantioselectivity, and tolerance to harsh conditions. Emphasis is placed on emerging molecular innovations and metagenomic approaches for discovering novel enzymes with enhanced industrial applicability. By bridging fundamental insights with practical applications, this overview aims to guide future research and development efforts in harnessing thermostable lipases for expanding biotechnological horizons.

INTRODUCTION

Thermostable lipases are extensively used in various biocatalytic reactions due to their excellent performance at elevated temperatures. Operating at high temperatures offers several advantages, including increased product formation rates with minimal diffusional limitations, improved conversion efficiency, enhanced solubility of hydrophobic substrates, greater molecular mobility, and a reduced risk of microbial contamination [1]. Lipases (EC 3.1.1.3, triacylglycerol acylhydrolases) rank among the most widely employed enzymes in organic synthesis and biotechnological processes. Although lipases from extremophiles exhibit activity across diverse pH ranges, their stability often declines above 70°C. Typically, microbial lipases show optimal activity between 30°C and 65°C. Extremophilic organisms inhabiting various environments have been identified as sources of a broad spectrum of thermostable lipases, whose stability under limited water conditions enhances their industrial applicability [2, 3]. Many thermostable lipases have been characterized from moderate extremophiles, particularly

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from the genus Bacillus, such as Bacillus sp. J-33 [4], *Lactobacillus acidophilus* [5], B*acillus thermoleovorans* ID1 [6], and *Pseudomonas aeruginosa* species [7]. Advances in genomics and protein engineering continue to deepen our understanding of thermostable lipases. These enzymes maintain exceptional stability in both aqueous and non-aqueous environments at elevated temperatures, facilitating higher reaction rates, reducing medium viscosity, and minimizing microbial contamination [1, 2]. Recent studies highlight structural features that contribute to their enhanced properties, including robust hydrogen bonding networks, increased hydrophobic interactions, and optimized surface loops—traits commonly observed in enzymes from extremophiles. For example, semi-rational design strategies have been used to improve thermostability, as demonstrated in novel esterase structures [8]. The exploration for novel thermostable lipases increasingly employs metagenomic approaches, enabling the discovery of enzymes from unculturable microorganisms in diverse extreme environments, such as medicinal wastewater [9, 10]. This has expanded opportunities to isolate wild strains with unique features and to engineer recombinant strains tailored to biotechnological needs. The intrinsic heat resistance of proteins in extremophiles arises from multiple molecular interactions that stabilize their structure, including strengthened ionic networks, enhanced hydrophobic contacts, increased hydrogen bonding, the presence of disulfide bridges, shortened surface loops, and the capping of N- and C-termini. Moreover, thermophilic enzymes exhibit a densely packed and rigid framework that supports stability at high temperatures but often compromises catalytic activity at lower temperatures. This reduced activity at moderate temperatures limits their application in processes where fine chemicals remain stable only under such conditions. Additionally, these biocatalysts can be sensitive to adverse factors like organic solvents, ionic strength variations, extreme pH, high pressure, and temperature fluctuations, which may challenge their economic viability in industrial processes [11]. Thermal Stability (T Max), Optimum pH, and half-life ($t_{1/2}$) of thermostable lipases from various naturally sourced microorganisms, illustrating their diverse stability profiles at different temperatures, are shown in Table 1.

Table 1: Thermal Stability, Optimum pH, and Half-Life of Thermostable Lipases From Various Naturally Sourced Microorganisms

Source	T max°C	pH optimum	Stability	References
Bacillaceae sp. RSJ1	50°C	8-9	$t_{1/2}$ = 150, 90, 55, 7 and 45 min at 60°C,65°C, 71°C and 76°C, respectively	[11]
Pseudomonas spp.	90°C	11	$t_{1/2}$ = 13 hr at 90°C	[12]
Bacillus thermoleovorans ID-1	70-75°C	7.5	$t_{1/2}$ = 30 min at 70°C and 1 hr at 60°C	[13]
Bacillus strain A30-1	60°C	6-9	$t_{1/2}$ = 8 hr at 75°C	[14]
AneurinIbacillus thermoaerophilus strain HZ	65°C	7	$t_{1/2}$ = 3 hr 10 min and 1 hr 20 min at 65°C and 70°C, respectively	[15]
Burkholderia multivorans V2	45-50°C	8	$t_{1/2}$ = ten and 50 minutes at fifty to sixty degrees Celsius, respectively	[16]
Burkholderia spp.	60°C	8.5	$t_{1/2}$ = 2 and 0.5 hr at 50°C and 60°C, respectively	[17]
Bacillus subtilis NS 8	60°C	7	$t_{1/2}$ = 4 hr 33 min at 60°C, 52 min at 71°C and ~42 min at 80°C	[18]
Bacillus coagulans MTCC-6375	45°C	8.5	$t_{1/2}$ = 4 hr 33 min at 60°C, 51 min at 70°C and ~42 min at 80°C	[19]
Geobacillus thermodenitrificans IBRL-nra	65°C	7	$t_{1/2}$ = 20 min at 55°C. 65°C 7 $t_{1/2}$ = 8 hr at 60°C, 16 hr at 65°C – 75°C	[20]

Optimum pH and temperature conditions for catalytic activity of thermostable lipases from various micro-organisms are shown in Table 2.

Table 2: Optimum pH and Temperature Conditions for Catalytic Activity of Thermostable Lipases from Various Microorganisms

Microorganism	Enzyme Optimum pH	Enzyme Optimum Temperature (°C)	References
Bacillaceae sp. RSJ-1	8-9	50°C	[11]
Bacillaceae acidocaldarius	-	68 to 70°C	[21]
Bacillaceae strain J33	8	60°C	[22]
Bacillus thermocatenulatus BTL2	9	60-70°C	[23]
Geobacillus Stearothermophilus	-	65°C	[24]
Geobacillus Stearothermophilus	8	70°C	[25]
Pseudomonas spp.	15	90°C	[26]
Pseudomonas sp.	9.7	60°C	[27]
Pyrobaculum calidifontis	-	95°C	[28]
Pyrococcus furiosus	-	99°C	[29]

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Pyrococcus horikoshii	7	95°C	[30]
Pyrococcus horikoshii	5.9	90°C	[31]
Staphylococcus aureus	5-12	55°C	[32]
Rhizopus chinensis	9	30-50°C	[33]
Thermoanaerobacter thermohydrosulfuricus	7-8	75-90°C	[34]
Staphylococcus xylosus	5-12	65-70°C	[35]
Bacillus Subtilis	10	55 to 79°C	[36]
Bacillus pumilus Rk31	7	40-70°C	[11]

Despite extensive studies on lipases from mesophilic and thermophilic sources, a consolidated understanding of the molecular determinants governing thermostability, catalytic efficiency, and industrial adaptability remains fragmented. Many reported thermostable lipases demonstrate promising activity under laboratory conditions; however, challenges persist in translating these enzymes into cost-effective, large-scale industrial applications due to limitations in production yield, solvent tolerance, and long-term operational stability. Furthermore, comparative analyses integrating structural insights, metagenomic discoveries, and enzyme engineering strategies are still limited. Therefore, a comprehensive review bridging molecular innovations with expanding industrial demands is essential to guide future biotechnological advancements.

Industrial Use of Thermostable Lipases

The application of substance catalysts doesn't just include many drawbacks linked to it; however as well provides an increase to several unwanted byproducts together with poisonous effluents, while at the same time, substrate uniqueness, bio-degradability, and lots of these kinds of positive aspects related to biocatalysts provide them with an advantage over the usage of catalysts in the chemical industrial sectors [37]. Thermostable enzymes can take on higher temperatures, therefore endowing a long half-life to the bio-catalyst. These types of enzymes may also put up with elevated levels of substrates as well as be protected from chemical denaturants. Their potential to perform different reactions at a high reaction rate as a result of a rise in substrate diffusion coefficient, as well as lower viscosity at elevated temperature ranges, make them a well-liked option over the sources of mesophiles [11]. The bio-technological possibilities related to lipases cause them to become a good biocatalyst within the world of many bio-technological and industrial sectors.

Application of Thermostable Lipases in the Food Industry

Lipases play a large role in the food industry in where they are widely used for manufacturing as well as customization of fats and oils (since they develop an essential element of food) to produce healthier food items. Lipases modify the lipids by causing the alteration within the placement of the important fatty acids in several glycerides [8]. The uniqueness related to lipases ensures they are a perfect option for oleochemical industrial sectors for the manufacturing of several higher value-added products, such as fats in human milk alternatives, the obroma oil counterparts, along with other specific prepared lipids [38]. Since absolutely no method provides such specificities, this amazing trait of thermostable lipases might be focused on industrial and commercial advancements.

Problem/Need

Lipases from *Thermomyces lanuginosus* happen to be identified by producing different transesterification and esterification reactions. Lipases from *Thermomyces lanuginosus* are recognized to perform the development of glycerides to be able to create healthier kinds of margarine [39]. Lipases seemed to be involved with the growth and development of several favouring agents. Thermostable lipases from several microbes have been employed to result in the oil customization, milk fat manufacturing similar to lipids, production of margarine making use of glycerides, and so on. Modification of fats and oils to produce healthier food products and speciality lipids requires enzymes with high specificity. Chemical catalysis often lacks positional specificity, generates unwanted byproducts, and cannot tailor fatty acid composition effectively.

How Thermostable Lipases Solve It

Thermostable lipases catalyze selective transesterification and esterification reactions, enabling precise alteration of fatty acid positions in glycerides at elevated temperatures, improving stability and reaction rates. This allows the creation of customized lipids, flavor development, and healthier fat substitutes [40].

Examples with Relevant Performance Data

Lipases from Thermomyces lanuginosus are recognized for producing modified glycerides used to make healthier margarine and for flavor generation. Beyond classic uses, thermostable lipases assist in synthesizing novel compounds such as mono- and di-acyl esters of glyceryl caffeate with enhanced antioxidant properties [3]. Their effectiveness at elevated temperatures (up to 70°C) supports industrial scalability.

Future Directions

Ongoing industrial innovation aims to improve thermostability and substrate specificity through enzyme engineering [41]. These advances will broaden the application scope in food biotechnology, enabling the production of novel functional lipids, customized fats for nutrition, and improved process efficiency.

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Detergent Sector and Thermostable Lipases

The cleaning agent market is among the major marketplaces for enzymes, along with a huge development that takes place to generate the newest enzymes, which include proteases, lipases, and amylases, along with greater as well as significantly better potential. The foremost commercially essential use of Thermostable lipases is within the cleaning agent business, where it's included with the laundry detergent for boosting the second activity [42]. Enzyme revenue in '95 was approximately thirty million dollars, with cleaning agent enzymes creating 30 per cent. The production of laundry detergents is approximately 13 billion tons per year, and the number of lipases is approximately 1,000. One of the primary problems in the laundry sector is the excretion of the adsorbed lipids through the components that are normally constructed of long-chain, water-insoluble triacylglycerols. These lipids might be eliminated by using cleaners containing lipolytic enzymes, which break them down into complementary fatty acids, diacylglycerols, and monoacylglycerols [43]. The First commercialized lipase used in cleaning agents was lipase TM, which Novo Nordisk introduced in the early nineteen nineties.

Problem/Need

Effective removal of water-insoluble, long-chain triacylglycerol lipid stains under harsh alkaline, high-temperature laundry conditions remains a challenge [44]. Conventional detergents lack sufficient enzymatic components to fully degrade these lipids, impacting cleaning performance and fabric care.

How Thermostable Lipases Solve It

Thermostable lipases hydrolyze complex triacylglycerols into free fatty acids and glycerides that are water-soluble and easily removed. Their stability at alkaline pH and resistance to temperature enable sustained catalytic activity during washing cycles, improving stain removal and preventing fabric greying.

Examples with Relevant Performance Data

Early commercial enzymes such as Lipase TM (originally from a thermophilic fungus, later produced in Aspergillus oryzae) and Lumafast™ lipases from Pseudomonas mendocina and P. alcaligenes demonstrated these properties [37]. Modern developments leverage metagenomic libraries to discover novel, thermostable lipases with enhanced detergent compatibility and stability.

Future Directions

Focused protein engineering and metagenomic screening aim to enhance lipase resistance to surfactants, oxidants, and thermal stress, driving development of detergents with superior cleaning power and reduced environmental impact [45].

Use of Thermostable Lipases in Drug Intermediates and Drugs

Thermostable lipases are also described because of their capability to give rise to the quality of several racemate blends of acids and alcohols [46]. Thermostable lipase, as a result of Humicola lanuginosa, has been referred to as among the most effective enzymes for contributing to the differentiation of two enantiomers artificially essential chiral foundation, (Z)-4-triphenyl methoxy-2, epoxy butan-1-ol, using ethylene-vinyl acetate as acyl donor. Lipases from *Thermomyces lanuginosus* demonstrate high enantioselectivity, as shown by their ability to hydrolyze complex bicyclic esters to yield optically pure compounds like (–)-epoxyalcohol and (–)-bromodiol. Additionally, Lipozyme TL IM (a commercial lipase from *T. lanuginosus*) has been effectively used for the regioselective acylation of 5′-O-acyl 5-fluorouracil 1-β-D-ribofuranoside, enhancing its antitumor potential compared to the parent drug 5-fluorouridine [47]. In pharmaceutical and drug intermediate synthesis, thermostable lipases are gaining prominence for their ability to catalyze chemo-, regio-, and enantioselective reactions, producing high-purity chiral compounds essential for drug development. Their robustness under various solvent conditions makes them ideal for complex organic synthesis routes.

Problem/Need

Stereoselective synthesis of optically pure drug intermediates demands regio-, chemo-, and enantioselective catalysis. Chemical synthesis often produces racemates, lowering drug efficacy and requiring costly purification steps.

How Thermostable Lipases Solve It

Thermostable lipases catalyze selective hydrolysis and acylation reactions even in organic solvents and at elevated temperatures, enabling the preparation of chiral intermediates with high purity and yield.

Examples with Relevant Performance Data

Lipases from Thermomyces lanuginosus achieve enantiomeric excess above 96% and purity greater than 99.5% in hydrolyzing bicyclic esters and paclitaxel precursors. Lipozyme TL IM catalyzes regioselective acylation of 5′-O-acyl 5-fluorouracil derivatives, enhancing antitumor potential [48]. Pseudomonas aeruginosa lipase demonstrates asymmetric hydrolysis of methoxy-phenyl glycidic acid methyl ester relevant to diltiazem synthesis.

Future Directions

Combining enzyme immobilization with protein engineering is expected to improve reusability, stability, and selectivity [49]. Exploration of thermophilic and extremophilic lipases may yield catalysts suited for broader pharmaceutical applications and more demanding synthesis conditions.

Environmental Applications and Biodegradation

The degradation of atmospheric macromolecules remains

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a significant challenge; however, this problem can be mitigated to some extent through the application of thermostable lipases. For instance, the side-chains of polyvinyl acetate were successfully hydrolyzed at 60°C in the presence of toluene using various lipases [50]. The efficiency of hydrolysis for long side-chains followed the order: hog pancreatic lipase > Novozym 435 > *Thermomyces lanuginosus lipase* (TLL) > *Candida rugosa* lipase. In contrast, the hydrolysis of shorter side-chains was initiated in the reverse order [40]. The deterioration of several different polymers as an illustration, poly (bisphenol) and polycaprolactone, has been produced at a specific temperature, which ranges between 27 and seventy degrees Celsius, using several lipases, for instance, lipases from hog-pancreas, *Thermomyces lanuginosus*, and Novozyme 436 (N 436) in the existence of different chemicals [10]. The highest temperature ranges noted for hog pancreas lipases, as well as others, respectively, had been around 50 and 60°C. However, the degradability perspective of lipase action was within the variety of *Thermomyces lanuginosus* > *Candida rugosa* > Novozym-435 > hog pancreas.

Problem/Need

Biodiesel synthesis requires efficient transesterification of lipids; cosmetic industries demand biocompatible, biodegradable polymers synthesized under mild conditions. Enzymes must sustain high temperatures and resist organic solvents [42].

How Thermostable Lipases Solve It

They catalyze lipid transesterification and ring-opening polymerization reactions at elevated temperatures (up to 80°C) and in organic solvents, enhancing process efficiency and product quality [50].

Examples with Relevant Performance Data

Lipase B from Candida antarctica and lipases from Thermomyces lanuginosus have shown exceptional stability and catalytic performance in biodiesel production and synthesis of polycaprolactone biopolymers used in cosmetics [41]. Their biocompatibility and permeability make them ideal for biomedical applications.

Limitations and Future Directions

This review is limited by its reliance on previously published experimental studies, as large-scale industrial validation data and long-term process optimization studies remain comparatively scarce. Variability in enzyme sources, expression systems, immobilization strategies, and assay conditions across studies makes direct comparison challenging. Protein engineering aims to broaden substrate specificity, improve solvent tolerance, and lower production costs [24]. Development of thermostable lipases with tailored catalytic properties will support sustainable biofuel manufacturing and next-generation cosmetic polymers.

CONCLUSION

Because the entire world is focusing on new developments every single day, along with the development in modern technology, the enzymes produced from thermophiles have acquired significant uses and therefore are a source of appeal for a lot of industrial sectors. Thermophilic lipases have grown in popularity during the past several years. Consequently, they are among the essential biocatalysts due to their remarkable capability to accomplish unique interfacial reactions as well as their capacity to catalyze changes. Due to their proficiency in experiencing unpleasant conditions, they've attained immense uses in the areas of bio-technology, pharmaceuticals, and microbiology. Checking up on all the innovative and exclusive characteristics of thermophiles, it's now essential to research completely new bacterial resources for all these enzymes and carefully look for their uses in different areas. Aside from this, methods such as molecular dynamics, as well as developing methods for free energy computation, allow us to obtain an understanding of the elements responsible for thermal resistance.

Authors' Contribution

Conceptualization: TB, TH
Methodology: A, SAB, TH, W, IR
Formal analysis: RB
Writing and Drafting: TB, TH
Review and Editing: TB, TH, A, SAB, TH, W, IR, RB

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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