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Use of genomics & proteomics in studying lipase producing microorganisms & its application

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ABSTRACT

In biotechnological applications, lipases are recognized as the most widely utilized and versatile enzymes, pivotal in biocatalytic processes, predominantly produced by various microbial species. Utilizing omics technology, natural sources can be meticulously screened to find microbial flora which are responsible for oil production. Lipases are versatile biocatalysts. They are used in a variety of bioconversion reactions and are receiving a lot of attention because of the quick development of enzyme technology and its usefulness in industrial operations. This article offers recent insights into microbial lipase sources, including fungi, bacteria, and yeast, alongside traditional and modern methods of purification such as precipitation, immunopurification and chromatographic separation. Additionally, it explores innovative methods like the reversed micellar system, aqueous two-phase system (ATPS), and aqueous two-phase flotation (ATPF). The article deals with the use of microbial lipases in a variety of sectors, including the food, textile, leather, cosmetics, paper, detergent, while also critically analyzing lipase-producing microbes. Moreover, it highlights the role of lipases in biosensors, biodiesel production, tea processing, bioremediation, and racemization. This review provides the concept of the use of omics technique in the mechanism of screening of microbial species those are capable of producing lipase and also find the potential applications.

1. Introduction

Enzymes are considered as biocatalysts and are found universally in living organisms. They are characterized by their remarkable precision in catalyzing specific reactions with minimal energy expenditure. They play a vital role in all metabolic and biochemical processes, with certain enzymes standing out for their applicability as catalysts in diverse biochemical reactions. Among these, lipases, a subclass of esterases, are particularly noteworthy for their involvement in the digestion, transport, and processing of lipids across various organisms (Ali et al., 2023). Lipases are incredibly versatile enzymes that help a lot of different bioconversion processes in both unicellular and multicellular animals, such as hydrolysis, alcoholysis, acidolysis, aminolysis, esterification, and interesterification. Their essential role lies in the bioconversion of triacylglycerols (TAG) within the organisms, enabling crucial metabolic processes and biochemical transformations (Ali et al., 2023). They also able to act on various types of functional lipids such ω-3 polyunsaturated fatty acids (Zhang et al., 2022). Lipases exhibit a unique trait of functioning at the interface between aqueous and non-aqueous phases, setting them apart from other esterases. They possess several distinctive properties, including pH sensitivity, temperature dependence, catalytic efficacy in organic solvents, and non-toxicity (Jaeger et al., 1998.). In addition to these, lipases are highly valued for their adaptability to different types of glycerides and free fatty acids in transesterification reactions, their durability in non-aqueous conditions, their low level of product inhibition, and their resistance to pH and temperature variations. It's noteworthy that lipases function normally and don't require cofactors to stay stable in organic solvent. In the realm of study, the microbial lipases have shown their significance (Roji et al., 2022).

Lipases can be used in the pharmaceutical industry, which has seen an increase in demand for enantiomerically pure drugs over the last 20 years (Jamilu et al., 2022). Remarkably, almost 80 percent of the

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medications created within this time frame contain chiral compounds. Lipase-catalyzed reactions, including carboxylic acid esterification, alcohol acylation, and ester hydrolysis, are a common feature of modern chiral drug production processes. (Carvalho et al., 2015). For instance, Tanabe Seiyaku used an immobilized lipase derived from *Serratia marcescens,* for the production of the anti-hypertensive Diltiazem in Japan. Similarly, the anticancer medication Taxol is made by Bristol-Myers-Squibb in the USA using an immobilised lipase from *Pseudomonas cepacia* (Godoy et al., 2022). Other examples include the preparation of (i) an intermediate of Duloxetine, a serotonin-norepinephrine reuptake inhibitor, by BASF in Germany, utilizing lipases from *Burkholderia plantarii* or *Pseudomonas* sp., and (ii) an intermediate of Crizotinib, a human hepatocyte growth factor receptor autophosphorylation inhibitor, by Agouron Pharmaceuticals in the USA, employing various commercial lipases (Krishna et al., 2002).

The search for new lipases continues because of the need to find enzymes with characteristics that make them competitive with their already-available commercial counterparts. These new microbial lipases are attainable via traditional methods involving the isolation of microorganisms from environments conducive to lipase production, followed by cultivation in laboratory settings. However, it's worth noting that merely a fraction, approximately 0.1 % to 1 %, of the bacterial species present in soil samples can successfully be isolated and cultivated under laboratory conditions (Torsvik et al., 1996).

Bacterial lipases are typically glycoproteins; however, certain extracellular bacterial lipases are lipoproteins (Abdul et al., 2013). The composition of the medium has a major influence on the production of extracellular lipases from bacteria, in addition to physicochemical characteristics like temperature, pH, and dissolved oxygen. Since lipases are induced enzymes, the primary determinant for the development of lipase activity has consistently been identified as the carbon supply. These enzymes are usually produced in the presence of a lipid, such as oil, or any other inducer, like bile salts, hydrolyzable esters, fatty acids, triacylglycerols, Tweens, and glycerol. However, essential micronutrients and nitrogen sources should also be carefully considered for growth and production optimization (Veerapagui et al., 2014).

Lipases can be employed in several chemical synthesis-related processes, including amidation, Michael addition, and aldol condensation (Dwivedee et al, 2018; Liu et al., 2001). However, the application of lipases in biocatalytic processes is rather low. The lipase from *Burkholderia cepacia* (Amano Lipase PS) and lipase B from *Candida antarctica* (CALB, Novozym 435 in its immobilized form) are two of the most utilized ones. These two commercial lipases are similar in a number of ways, such as their ability to catalyse a large range of reactions with highly selective activities, their stability and good activity in organic solvents, and their stability over a broad pH and temperature range. Moreover, they show notable enantioselectivity. It has been observed that lipase from *Burkholderia cepacia* show specificity to vinyl 4-phenylbutyrate and also exhibited excelled characteristic to form 3′-arylaliphatic acid esters of 5-fluoro-2′-deoxyuridine (Li et al., 2009). It has been further observed that the lipolytic activity of lipase from *Burkholderia cepacia* showed lipolytic activity higher than any other types of lipase available (Dulęba Jacek, Siódmiak Tomasz, Marszałł Piotr Michał, Amano Lipase PS from *Burkholderia cepacia* – Evaluation of the Effect of Substrates and Reaction Media on the Catalytic Activity, Current Organic Chemistry; Volume 24, Issue 7, Year 2020). Studies have also showed that the immobilized *Candida Antarctica* lipase showed enhanced activity in comparison to the immobilized lipase (Wu et al. 2023).

The goal of the ongoing search for new lipases is to identify those with characteristics that will enable them to compete with the lipases that are currently on the market. Novel lipases can be produced by the so-called "traditional" techniques of growing the bacteria in a lab and removing microorganisms from environments that are favourable to lipase makers. Nevertheless, it is only possible to isolate and grow in a lab 0.1 % to 1 % of the bacterial species found in soil samples (Torsvik et al., 1996). Some of the factors that contribute to this situation are the interdependence of the species, the existence of substances that hinder growth, the organism's transient low metabolic activity, the absence of a surface that is conducive to growth, and the incorrect combination of temperature, pH, pressure, water activity, gas phase composition, and nutrients.

One approach to solving these issues is the investigation of biodiversity via omics science (Handelsman et al., 2004). Samples are taken from locations thought to harbor lipase-producing microbes for an omics search for novel lipases. DNA fragments found in these samples are then extracted, sequenced, cloned, and produced in a heterologous host, typically *Escherichia coli*. It is possible to screen the clones for lipases as well as other relevant enzymes or metabolites.

To circumvent challenges such as the presence of inhibitory substances, temporary low metabolic activity of organisms, lack of suitable growth surfaces, and suboptimal environmental conditions like temperature, pH, pressure, water activity, gas phase composition, and nutrients, Consequently, metagenomics' investigation of biodiversity gives a promising solution. In this approach, samples are collected from environments rich in potential lipase-producing microorganisms, and DNA fragments within these samples are isolated, sequenced, cloned, and expressed in a heterologous host, typically *Escherichia coli*. These clones are then screened for lipases as well as other enzymes or metabolites of interest (Vartoukian, 2010).

For humankind, lipases are essential to the manufacturing and service industries. Microbial lipases have drawn particular interest from the industrial sector because of their broad substrate specificity, stability, and selectivity. Production of microbial enzymes is safer and more convenient. Additionally, compared to the corresponding plant and animal enzymes, they are more stable (Bharathi and Rajalakshmi 2019) (Table 1, Fig. 1).

Properties of lipases typically exhibit molecular weights ranging from 19 to 60 kDa and are known to exist as monomeric proteins. The length, degree of unsaturation, and location of the fatty acid in the glycerol backbone are some of the factors that affect the physical properties of lipases. These factors collectively contribute to the variability observed in the characteristics of lipases (Carpen et al., 2019). Additionally, these characteristics affect a specific triglyceride's sensory and nutritional qualities. Apart from that, a number of lipases also show activity in organic solvents, which allows them to catalyze many helpful processes including esterification. This versatility underscores the significance of lipases in various industrial processes and applications (Las et al., 2018). Lipases exhibit pH-dependent activities, typically showing optimal activity around neutral pH 7.0. Lipases can remain stable within a pH range of 4.0–8.0. Examples include extracellular lipases from *Chromobacterium viscosum*, *A. niger*, and *Rhizophus* sp., which demonstrate activity at acidic pH levels. Conversely, lipases produced by *P. nitroaeducens* are alkaline in nature and exhibit optimal activity at pH 11.0 (Abreu et al., 2017). In specific experimental circumstances, lipases possess the capability to catalyze reversible reactions, allowing for both esterification and interesterification processes to occur even in the absence of water. This unique characteristic highlights the versatility of lipases as biocatalysts and expands their potential applications in various industries (Ekinci et al., 2016). Lipase activities are significantly inhibited by Co, Ni^{2+}, Hg^{2+}, and Sn^{2+}, while Zn^{2+}, Mg^{2+}, EDTA, and SDS cause slight inhibition. Temperature stability profiles reveal that lipases exhibit longer half-life values at lower temperatures, indicating greater stability. Region-specificity of lipases leads to their classification into two groups, which is determined by the acyl glycerol substrate. In the first group, lipases exhibit regiospecificity and release fatty acids exclusively from all three positions of glycerols (Gaschler et al., 2019). Fatty acids are released from the 1 and 3 positions of acylglycerols in a region-specific manner by the second set of lipases. These lipases hydrolyze triacylglycerols to produce 1,2-(2,3)-diacylglycerols, 2-monoacylglycerols, and free fatty acids. Partial stereo-specificity has been observed in the hydrolysis of triacylglycerols by lipases from *A. arrhizus*,

Table 1
Various microbial species responsible for the production of lipase.

Microbial Source	Isolated from	Applications	Reference
Thermomyces lanuginosus	Hull-less pumpkin oil	Sustainable production of lipase	Darjijo et al., 2020
Thermomyces lanuginosus, Candida antarctica	geraniol and citronellol	Artificial flavour esters produced by enzymatic catalysis.	Da Silva et al., 2020
Nannochloropsis oculata	Marine source	Production of sustainable Biodisel	He et al., 2020
Candida antartica	Cooking oil	Production of Diacylglycerol	Razak et al., 2020
Rhodococcus sp	Sunflower oil	Production of lipase	Maniyam et al., 2020
Rhodococcus sp	Waste cooking oil	Development of glycerol	Maegala et al., 2020
Anoxybacillus gonensis UF7	Waste frying oil	Amoxylipase is used for the production of biodiesel.	Altinok et al., 2023
Bacillus amyloliquefaciens	Agro-Industrial Residues	Enhanced production of lipase in semi-solid fermentation	Mazhar et al., 2023
Bacillus spp	Abattoir soil	It showed positive lipase activity at the end of period of 24 h.	Mathew et al., 2023
Leptospirillum spp.	Acidic Environment	Possess the ability to survive at low pH and are involved in different industrial processes.	Vergara et al., 2020
Acidithiobacillus ferrooxidans	Acidophilic and chemo lithophilic sulphur and iron oxidising bacteria.	Is used for the process of biobleaching in extraction of metal.	Gao et al., 2020
Escherichia coli	From acidic conditions	Possess the ability to grow in moderate acidic environment	Xu et al., 2021
Exiguobacterium sp.	Water samples from glaciers	Helps in the development of biodetergent formulation	Yasin et al., 2021
Serratia nematodiphilia	Pulp and paper effluent	Helps in deinking process of the papers.	Intwala and Barot 2022
Enterobacter cloacae	Acidic conditions	It has potential pharmaceutical use	Asitok et al., 2023
Hansenula anomala	Fermented form of soyabean	Helps in excellent production of lipase.	Cui et al., 2020
Geotrichum candidum	Milk of Tibet kefir	Help in removing 99 % of Pb^{2+} ions.	Meng et al., 2020
Brettanomyces spp	Products associated with bewing	Helps in adding new flavour to craft beer.	Colomer et al., 2019
Trichosporon spp	Biomass of lignocellulose	Helps in the production of ethanol under anaerobic condition.	Yu et al., 2020
Penicillium simplicissimum	Food samples	Increase the production of lipase in the presence of olive oil. It can be used in various types of food industries.	Greco-Duarte et al. 2023
Penicillium roqueforti	Organic samples	Used for the synthesis of ethyl oleate	Soares et al.' 2022

R. delemar, C. cylindracea, and *P. aeruginosa* (Nurhasanah et al., 2020). The increase in demand for the use of sustainable and efficient biocatalysts, coupled with the limitations in the use of traditional methods in the field of lipase research resulted in the understanding of various lipase producing microorganisms at its molecular level. The use of the knowledge of genomics and proteomics integrated together resulted in better understanding of the various microbes those are responsible for the production of the enzymes. Thus this review focussed on the various lipase producing microbes, recent state of art-techniques in identification and lastly the potential applications of the enzyme. Thus the novelty of this review stands with the integration of various latest technologies in studying lipase along with the lipase producing organisms.

2. Kinetic model for lipolytic activity

The Michaelis-Menten model is unable to adequately describe lipolysis that occurs at the substrate/water interface. It has been observed that in homogeneous phase the lipase is effective as biocatalysts (Sarkar et al., 2019) that can be predicted by the use of various simple models comprising of two consecutive equilibrium (Fig. 2) (Hitaishi et al., 2018).

In the process of two phase lipase reaction system comprising of aqueous and organic solvent phase in which the lipid remains dissolved within the organic phase whereas the lipase remains dissolved within the aqueous buffer. Thus the reaction take place at the interface between the interface of organic and aqueous solution. The rate of the reaction can be derived in the following manner:

It is assumed that at initial interface the reaction follows Michaelis-Menten Kinetics

$$\mathbf{E+S} \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{K_{+2}} \mathbf{E+P} \quad (1)$$

In this model of reaction of the concentration of ES complex was considered to be constant. Whereas [E]n, [S]n and [ES]n are the concentration of enzyme, substrate and enzyme substrate complex at the interface. Therefor the rate of cahege of [ES] complex will be

$$d/dt(ES)_i = k_{+1}[E]_i[S]_i - (k_{-1} + k_{+1})[ES]I \quad (2)$$

Thus to simplify the mechanism of reaction, it is considered that the concentration of enzyme and substrate at the interface i.e. both in oranic and iorgaic phase, are equal.

The enzyme's alterable adsorption to the interface (E↔E*) takes place during the initial equilibrium phase. Within this phase, a lone substrate molecule (S) binds with the adsorbed enzyme (E*), resulting in the formation of the (ES) complex. This interaction leads to the subsequent phase of equilibrium (Khodayari et al., 2014). This latter equilibrium mirrors the Michaelis-Menten equilibrium for the enzyme-substrate complex. Following this, the products are released, and the enzyme is regenerated into the initial form (E). This renewal initiates the subsequent catalytic steps once the (E*S) complex is formed (Bansode et al., 2017). The lipase adsorbed in the vicinity of the substrate concentration at the interface is quantified in terms of surface concentration, as opposed to the conventional volumetric concentration in the atmosphere (Hanefeld et al., 2009). The rejuvenated lipase remains attached to the interface and is only liberated after undergoing numerous catalytic cycles in this model.

The activity of lipase is shaped by the interfacial conformation: the enzyme's behavior can vary, including denaturation, activation, or neutralization. The interface serves as a convenient location for modulating lipolysis. Direct interaction of lipase inhibitors with the enzyme impedes its activity. Conversely, certain compounds can postpone the lipolytic reaction through either adsorption to the interphase or to the substrate molecules (Birari et al., 2007). There are two primary types of lipase inhibitors: (a) synthetic lipase inhibitors, which include phosphonates, boronic acids, and fat analogues; and (b) natural compounds, which include β-lactones and different plant foodstuffs, such as plant extracts and metabolites, which primarily consist of polyphenols, saponins, peptides, and particular nutritive fibres. Lipases are essential for the absorption of lipids; therefore, lipase suppression is a useful strategy for managing obesity or the absorption of fat. β-lactones, including orlistat, represent natural compounds known for their capacity to inhibit lipase activity (Bialecka et al., 2018). Pancreatic lipase is accountable for breaking down over 80 % of total dietary fats. Orlistat, a registered drug in several countries, is utilized for treating obesity (Drent et al., 1993).

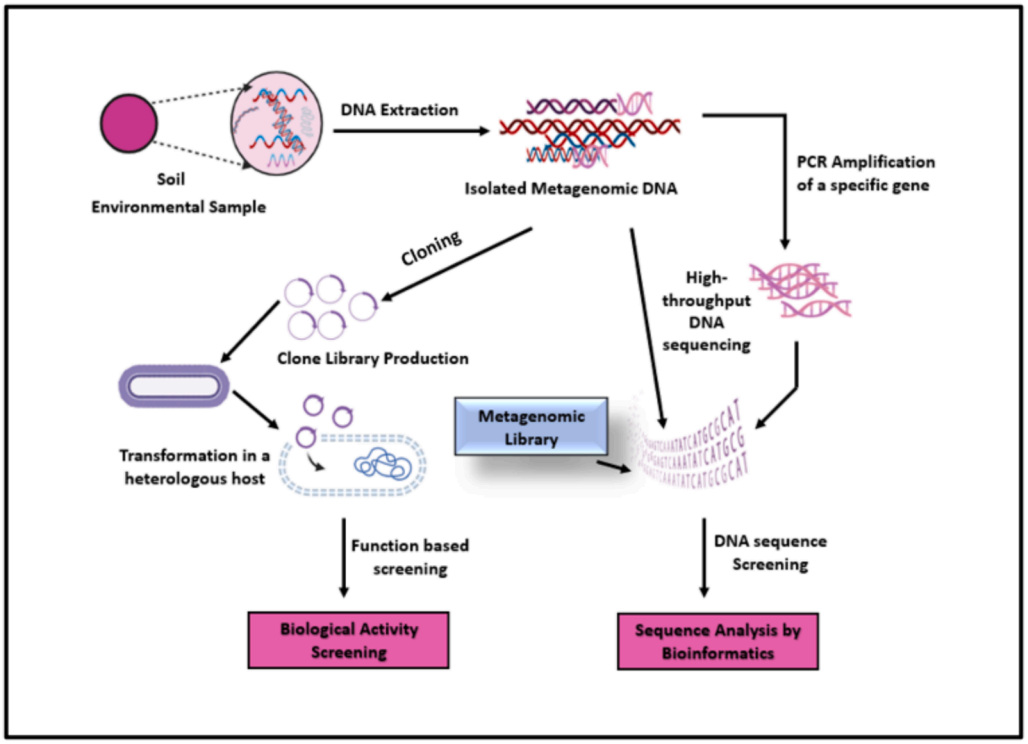

Fig. 1. The general process of using the metagenomic technique to isolate and identify new genes and enzymes from samples taken from the environment (Contesini et al., 2020).

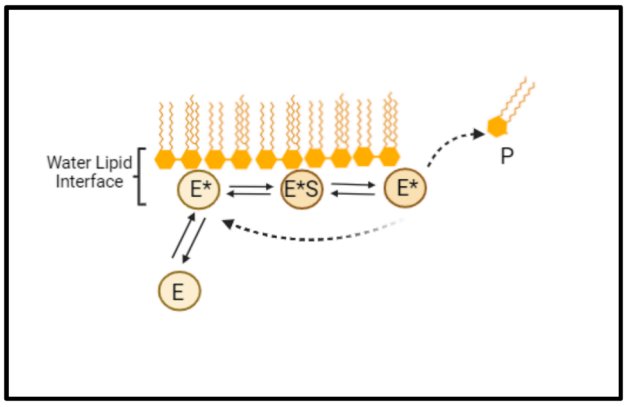

Fig. 2. Kinetic model for lipolytic activity.

3. Chemistry of lipase activity

There are five steps involved in the hydrolysis of esters. Hydrolysis requires the formation of a hydrogen bond between an ester of carboxylic acid and the residues of the oxyanion hole. It forms the Michaelis complex. When the enzyme is at rest, the side chain hydroxyl proton of the active serine is a part of the hydrogen bonding network that forms the catalytic triad. The oxygen in serine thus becomes more nucleophilic and is able to target the sp2-hybridized carbon atom in the substrate, which will ultimately hybridise into sp3. The ester oxygen forms H-bonds with different amino acid residues in the oxyanion hole, stabilising the negative charge of the serine. The catalytic histidine's subsequent positive charge is stabilised by the active sites, aspartate or glutamate. As a result, the original tetrahedral transition state is formed. The initial tetrahedral transition state of the hydrolysis reaction is assumed to be the rate-limiting step.

When the enzyme is at rest, the side chain hydroxyl proton of the active serine is a part of the hydrogen bonding network that forms the catalytic triad. Consequently, the oxygen in serine becomes more nucleophilic and can target the sp2-hybridized carbon atom in the substrate, which will finally hybridize into sp3. Through the formation of H-bonds with different amino acid residues in the oxyanion hole, the ester oxygen stabilizes the negative charge of the serine.

The catalytic histidine's subsequent positive charge is stabilized by the active sites, aspartate or glutamate. As a consequence, the original tetrahedral transition state is formed. The initial tetrahedral transition state of the hydrolysis reaction is assumed to be the rate-limiting step. As catalysts in numerous industrial applications, lipases derived from microorganisms, including bacteria and fungi, have drawn significant interest, including ester synthesis, optical resolution, transesterification, and washing processes (Xiao et al., 2015).

4. Production of microbial lipases from agricultural wastes

Microorganisms that generate a diverse range of extracellular lipases are typically the source of commercially valuable lipases. Merely 2 % of the global microbiome has undergone testing as a source of enzymes. Solid-state fermentation techniques can also be utilized to manufacture microbial lipases; however, submerged culture is the primary approach (NIAM research report, 2012). Solid-state fermentation is generally more cost-effective and well-suited than submerged fermentation for producing a variety of bioproducts, including animal feed, enzymes, organic acids, chemicals with aroma, antibiotics, compost, biopesticide, and biofertilizers. One high-recovery technique for producing industrial enzymes is solid-state fermentation (Benjamin et al., 2014).

There are concerns about the economic feasibility of substituting enzymatic procedures for chemical methods, despite the consensus that enzymatic processes are more environmentally friendly than traditional processes (Mahadik et al., 2002; Kamini et al., 2000). Because of their accessibility, affordability, and high nutrient content, agricultural

leftovers will therefore be more heavily utilized in future biotechnologies for the manufacturing of enzymes and other value-added products (Chugh et al., 2010). The employment of various microorganisms, supplements, and substrates to find the optimal combinations for high-value lipases under operating circumstances that provide cheap production costs at industrial scale is now one of the key areas of lipase research (Abdullah et al., 2022).

According to Alonso et al. (2005), industrial waste and its complex organic contents are an important source of residual nutrients that act as rich media for microbial growth and enzyme activity. Bacterial lipase has been produced utilizing solid-state fermentation and waste from the oil and agricultural industries. Numerous studies indicate that physical factors like temperature, pH, and heat conductivity—as well as the characteristics of the solid substrate, such as particle size and water activity have a significant influence on the amount of lipase that is produced from these wastes (O'Donnell et al. 2020). It has been observed that the activity of crude lipase enzyme from *Pseudomonas aeruginosa* at pH 6.5 and at a temperature of 35 °C (Teeragawinsakul and Siwarungson 2001).

Some of the biochemical properties of a crude lipase enzyme from Rhizopus arrhizus were determined. The enzyme displayed maximal catalytic activity at pH 9.0 and temperature of 35 °C. It was stable at basic values of pH and 40 °C and in the presence of detergents. Lipase showed substrate specificity against different plant oils.

Agricultural leftovers are mostly used as landfills and animal feed, despite being produced in vast quantities in poor nations (Chugh et al., 2010). Approximately 25 % of the fruits and vegetables produced in India, the world's second-largest producer, are lost during harvest. It is noteworthy that in industrialized nations, losses only transpire at the retail and consumer levels; in contrast, forty percent of losses occur at the post-harvest and processing stages (Xing et al., 2012). Agricultural wastes have been utilized in biotechnological processes recently, including the synthesis of chemicals with added value and substrates for microbial isolation.

These assist with issues related to disposal that might otherwise result in contamination (Banjamin et al., 2014). Because the remaining oil content acts as an inducer for lipase formation, oil cakes from various oil extraction businesses have been used for fermentative lipase production (Chugh et al., 2010).

A powerful substrate for *Candida rugosa* derived lipase synthesis during solid-state fermentation is coconut cake (Choi et al., 2014). *Aspergillus sp*. (Madhavan et al., 2017) and *Rhizopus sp*. were cultivated to produce high amounts of lipase. Cheap agricultural byproducts like olive oil cake and gingelly oil cake (Uchiyama et al., 2005) have recently attracted a lot of attention as viable substrates for solid-state fungal fermentation. According to Chugh et al., mold strains were chosen to produce enzymes on solid substrates. Gum Arabic can improve the availability of lipid substrates, which in turn can increase the synthesis of enzymes (Peña-García et al., 2016). According to Ramachandran et al, a possible crude material for amylase synthesis is coconut oil cake. According to Chugh et al. (2010) the most common lipid substrate for encouraging bacteria to produce lipase is olive oil. According to Elibol and Ozer (2001), the pH of the culture, the temperature at which it grows, the kind and concentration of carbon and nitrogen sources, and the concentration of dissolved oxygen all affect lipase synthesis. *Candida rugosa* can produce more lipase by a unique technique called mixed solid substrate fermentation (Choi et al., 2014).

Microbial source Lipases are abundant in microorganisms, spanning bacteria, yeasts, and filamentous fungi. In contrast to lipases derived from animals, such as pancreatic lipases, and plants, like those from Carica papaya and seeds, microbial lipases have been extensively utilized owing to their compatibility with simple and cost-effective culture media, ease of handling, scalability of cultivations, and the availability of diverse tools for genetic and protein engineering. The profound understanding of their genetics and physiology, particularly for model organisms such as *E. coli, K. phaffii*, and *Saccharomyces cerevisiae*, positions them as pivotal candidates for lipase production (Sarmah et al., 2018).

The majority of investigated lipases originate from bacterial sources, encompassing enzymes from various genera such as *Bacillus*, *Geobacillus*, *Pseudomonas*, *Streptomyces*, *Burkholderia, Chromobacterium, Achromobacter, Arthobacter,* and *Alcaligenes* (Samoylova et al., 2019). Among these, *Bacillus* lipases have been extensively studied, demonstrating stable activity at elevated temperatures across a wide pH range, in addition to their resilience to organic solvents (Sarmah et al., 2018;). However, from an industrial standpoint, lipases produced by yeasts and filamentous fungi are more appealing due to their capacity to be obtained in high concentrations and their distinctive properties concerning thermal stability and substrate specificity (Mehta et al., 2017). Fungal lipases from Candida, Geotrichum, Trichosporon, Yarrowia, Aspergillus, Penicillium, Rhizopus, Rhizomucor, and Thermomyces are particularly notable (Borrelli et al., 2015).

Microorganisms thriving in extreme environments, characterized by extreme pH, salinity, and temperature conditions, serve as crucial sources of industrial lipases. However, these microorganisms might be challenging to propagate under laboratory environments, making their lipases harder for human use. Extremophile bacteria, including thermophiles and psychrophiles, found in harsh environments such as hot springs, deep-sea sediments, and polar regions like Antarctica, possess the ability to survive in such hostile conditions and create a variety of lipases with distinct tolerance characteristics. Extremophile lipases have been the subject of numerous studies (Samoylova et al., 2018). There are now 1364 metagenomics studies of environmental samples covering more than 10,000 microorganisms, according to the Genomes OnLine Database. (https://gold.jgi.doe.gov/). These information underscore that Uncultivated microbial communities have enormous potential as sources of novel lipases with catalytic activity for a variety of substrates, along with pH and temperature tolerance. We expect that in the future, innovative industrially relevant lipases will be produced by rigorously screening codon-optimized lipase genes from various sources in heterologous hosts.

5. Use of recombinant technology in lipase modification

Homologous expression becomes a feasible alternative once the naturally produced lipase can be effectively propagated inside a bioreactor. In such cases, the mechanism tends to be more straightforward as lipase synthesis might already be naturally optimised. However, there is often a desire for higher lipase yields, which can be achieved through various strategies. One approach involves using powerfull promoters to drive the expression of the lipase producing gene. Additionally, the host organism can be changed to contain more copies of the lipase gene, either by directly inserting lipase genes into the genome or through the use of plasmids carrying the genes. Several studies have explored the production of recombinant lipases through homologous expression, including in bacteria such as *Serratia marcescens* (Chen et al., 2017) and *Burkholderia cepacia*, as well as in the filamentous fungus *A. niger* (Zhu et al., 2020).

Nevertheless, given the technological demands of the scale-up process—such as quick growth, maximal protein output, and the optimum physiological characteristics requires in bioreactors—natural lipases might not be appropriate for industrial use. Heterologous expression, which employs reliable hosts, is therefore an alternative. Technically speaking, heterologous expression systems consist of 3 main processes: (i) cloning of the desired gene into a vector containing a selection marker; (ii) transforming the host species using the plasmid that was created; (iii) expressing the gene of interest with the help of a known terminator and an inducible or constitutive promoter. The biological systems used for gene expression include prokaryotic and eukaryotic hosts, such as *E. coli* and *K. phaffi*, respectively (Samoylova et al., 2019). Several studies has chosen heterologous expression system to produce recombinant lipases from uncultured bacteria from harsh environmental

conditions. Their lipases encoding genes can be isolated and expressed in heterologous expression systems by the construction of functional metagenomics libraries (Yang et al., 2017). Functional metagenomics does not require either individual genome sequencing or cultivating unknown producer microbes, this leads to a huge advantageous situation. For more metagenomics studies of lipases with biotechnological potential, Almeida and collaborators (Almeida et al., 2020) provide an interesting review. It is observed that the regulatory mechanisms governing lipase biosynthesis varied significantly amongst microbes. In the case of *Calvatia* (Christakopoulos et al., 1992), *Rhizopus* (Salleh et al., 1993), *Aspergillus* (Pokorny et al., 1994), and *Rhodotorula* (Papaparaskevas et al., 1992), apparently, lipase production is constitutive and independent of the addition of lipids, despite the fact that their presence enhanced the levels of lipase produced. Conversely, the literature suggests that long-chain fatty acids – for instance oleic acid – participate in lipase expression in *Geotrichum candidum*, by controlling induction in the transcription level (Shimada et al., 1992). Concerning carbon sources, in *Fusarium* sp., carbohydrates have been described as repressors of lipase production (Rapp et al., 1995). Interestingly, in terms of comparison, the expression levels of lipase genes in some organisms, including *K. phaffii* and *Aspergillus*, can be hundreds of times higher than that in the native host, resulting in levels even greater than grams per liter (Jahic et al., 2003). To achieve economically viable productivity yields, strains engineered for overproduction of recombinant lipases should also have their fermentation conditions optimised. The most important variables are pH, temperature, agitation, oxygen, nutrients (salts and sources of carbon and nitrogen), and oxygen. In recent decades, the use of multivariate analytic tools—i.e., response surface methodology (RSM), has allowed for the optimization of lipase production by investigating different parameters simultaneously, which allows higher yield levels compared to univariable studies (Contesini et al., 2009). The scientists evaluated various media and found that the highest amount of extracellular lipase was obtained when *E. coli* was grown in Nutrient Broth. A lipase from *Bacillus* sp. was generated heterologously in *E. coli*. The addition of surfactants, which may aid lipase in remaining soluble even at high concentrations, further increased lipase's influence. The combination of optimized host, vector, surfactant and media resulted into an 18-times increase in production of lipase (214 units/mL) (Khurana et al., 2017). In another study, researchers investigated the ideal conditions for production of lipase from *Acinetobacter haemolyticus*, in *E. coli*. The authors observed a 70 % improvement using the following optimal conditions: OD600 equal to 0.6 (before induction), IPTG (Isopropyl β-d-1-thiogalactopyranoside) equal to 0.5 mmol/L, post-induction temperature equal to 40 ∘C, and post induction time equal to 16 h (Batumalaie et al., 2018). An excellent way to cut lipase production costs is to use inexpensive ingredients, primarily agricultural and industrial wastes, in recombinant lipase production. Nooh et al. (2018) optimized the production of a lipase from *Geobacillus* sp. After being cultivated in cheap substrates with RSM, in *E coli*. With 1.0 g/L of molasses, 2.29 g/L of fish waste, 3.46 g/L of NaCl, and 0.03 mM of IPTG, a maximal activity of 164.37 U/mL was attained. For recombinant lipase production to be scaled up, fermentation modes are just as crucial as the nutritional composition of the growth media. They consist of continuous, fed-batch, and batch cultivation techniques.

Robert et al. assessed fed-batch operational modes for producing the recombinant lipase B from *Candida antarctica* under the constitutive promoter PGK in *K. phaffii*. More specifically, the continuous mode was proven to be more effective in the long run.

However, native lipases can't always match technical criterias of industrial applications, such as rapid growth, maximal protein production, or ideal physiological parameters required in bioreactors. Consequently, heterologous expression emerges as an alternative, utilizing efficient host organisms. Heterologous expression systems typically involve three main steps: (i) cloning of the targeted gene into a vector which has a selection marker, (ii) transforming the host strain with the engineered plasmid, and (iii) expressing the gene of interest under the control of a constitutive or inducible promoter and a well-known terminator. Prokaryotic and eukaryotic hosts, such as *E. coli* and *K. phaffi*, respectively, are commonly employed for gene expression (Samoylova et al., 2019).

Heterologous expression has also been employed in several studies investigating recombinant lipases derived from Microorganisms that have never been grown and live in harsh environments. The genes encoding their lipases can be isolated and expressed in heterologous systems through the construction of functional metagenomics libraries (Yang et al., 2017). Functional metagenomics has the advantage of avoiding individual genome sequencing and the growth of unknown producer microbes. Almeida and colleagues provide a comprehensive review of metagenomics studies exploring lipases with biotechnological potential (Almeida et al., 2020).

Regarding the regulatory mechanisms of lipase biosynthesis, there is considerable variation among different microorganisms. For instance, lipase production appears to be constitutive and independent of lipid addition in certain organisms such as *Calvatia, Rhizopus, Aspergillus*, and *Rhodotorula* (Tang et al., 2019). Conversely, in *Geotrichum candidum*, long-chain fatty acids, like oleic acid, seem to regulate lipase expression at the transcriptional level (Shimada et al., 1992). Carbon sources have also been implicated in regulating lipase production, with carbohydrates described as repressors of lipase production in *Fusarium* sp. (Rapp et al., 1995).

In terms of expression levels, some organisms, including *K. phaffii* and *Aspergillus*, exhibit significantly higher expression levels of lipase genes compared to native hosts, resulting in levels exceeding grams per liter (Brunel et al., 2004).

Optimizing fermentation conditions is essential for achieving cost-effective yields of recombinant lipase production. Key parameters include temperature, oxygen, pH, nutrients like C and N sources and salts, and agitation. Response surface methodology (RSM) has proven valuable in optimizing lipase production by investigating multiple parameters simultaneously, leading to higher yield levels compared to single-variable studies (Contesini et al., 2009).

For instance, a lipase from *Bacillus* sp. was generated heterologously in *E. coli*, with optimized fermentation conditions resulted into an 18-times increase in production of lipase. Another study focused on optimizing conditions for producing an *Acinetobacter haemolyticus* lipase in *E. coli*, achieving a 70 % improvement in production (Khurana et al., 2017).

For recombinant lipase production, utilizing low-cost substrates, particularly wastes from agro-industries, could significantly reduce production costs. Nooh and colleagues achieved maximum lipase activity using low-cost substrates in *E. coli* cultivation (Nooh et al., 2018).

Furthermore, numerous fermentation methods, which includes batch, fed-batch, and continuous cultivation systems, play a crucial role in scaling up recombinant lipase production. Continuous cultivation has been shown to be particularly effective in producing recombinant lipase B from *Candida antarctica* under a constitutive promoter in *K. phaffii* (Robert et al., 2019) (Table 2).

6. Use of eukaryotic expression system

Lipases are mostly derived from eukaryotic expression systems, including the filamentous fungus *Aspergillus* and the yeasts *K. phaffii*, *S. cerevisiae*, and *Y. lipolytica* (Borrelli et al., 2015). Several of these microbes are widely acknowledged as safe (GRAS) (Robinson et al., 2017) and are effective producers. A variety of post-translational modifications (PTMs) (Robinson et al., 2017) can alter the chemical and physical characteristics of proteins made in eukaryotic cells, making them more stable, secretable, and soluble. Industry interest in fungi lipases has grown as a result of their substrate selectivity and stability in a variety of environments.

Methylotrophic yeast *K. phaffii* can express and generate lipases in response to methanol-responsive alcohol oxidase promoters. Its

Table 2
Recombinant Lipase production.

Name of the Enzyme	Source of the Enzyme	Name of Expression Vector	Name of Cloning vector	Molecular Mass	Optimum pH	Thermal Stability	Remarks	Reference
Lipase BaG7Lip	*Bacillus amyloliquefaciens* G7	*E. coli* BL21 (DE3)	*E. coli* BL21-Star (DE3)(p15TV-L)	26	50 °C, 8.0	85 % at 50 °C, 250 min	Build metagenomics libraries and use a Boolean network analysis to forecast the optimal production conditions. Acetone, glycerol, and K^+ ions are used to promote activity.	Khan et al., 2020
Lipase KV1	*Acinetobacter haemolyticus*	*E. coli* BL21 (DE3)	*E. coli* JM109 (pGEM-T Easy)	39	40 °C, 8.0	$T_{1/2}$–40 °C, 24 h	response surface methodology (RSM)-based production condition optimisation	Batumalaie et al., 2018
Cholesterol esterase + foldase	*Burkholderia stabilis* FERMP-21014	*E. coli* BL21 (DE3), *E. coli* Rosetta (DE3) and *B. stabilis*	*E. coli* DH5α and JM109	–	–	–	RNA-Seq promoter screening combined with foldase and lipase co-expression as well as homologous and heterologous expression. The *B. stabilis* system produced esterase more effectively than the WT without oleic acid, resulting in a 243-fold increase in recombinant activity.	Yoshida et al., 2019
Lipase LipBC (LipA) + foldase LifBC (LipB)	*Burkholderia contaminans* LTEB11	*E. coli* BL21 (DE3)	_	36 (LipA), 37 (LipB) and 66 (complex LipA–LipB)	25–45 °C, 6.5–10.0	$T_{1/2}$–50 °C, 1.5 h	chaperone and lipase gene co-expression. 1426 U/mg of specific activity, at 45 °C.	Alnoch et al., 2018
Lipase LipBT and foldase LifBT	*Burkholderia territorii* GP3	*E. coli* DH5α, *E. coli* DH10β, *E. coli* BL21 (DE3) pLysS, *E. coli* Origami B, *E. coli* Shuffle B, and *E. coli* SHuffle K	pGEM-T Easy (*E. coli* DH5α), pET15b (*E. coli* DH10β)	30	80 °C, 11.0	$T_{1/2}$–70 °C, 30 min, pH 8.0	Metagenomics for lipolytic strain discovery and screening + assessment of optimal expression systems. Improved activity in n-hexane, Triton X100, and Ca^{2+} and Mg2 + ions; higher lipase activity in *E. Coli* BL21 (DE3) pLysS (pET15b) (6.73 ± 0.24 U/mg); ideal substrate pNP-C10.	Putra et al., 2019
Lipase HT1-5M	*Geobacillus zalihae*	*E. coli* BL21 (DE3) pLysS	pUC57 and pGEX-4 T1 (*E. coli* TOP10)	44	70 °C, 9.0	Stable at 30–60 °C for 30 min	Molecular dynamics (MD) combined with site-directed mutagenesis and rational design. Increased activity when combined with Ca^{2+} ions; more stable when combined with DMSO, n-hexane, and n-heptane.	Ishak et al., 2019
Lipase Lip3	*Drosophila melanogaster*	*E. coli* BL21 (DE3)	–	43	–	$T_{1/2}$–37.3 °C (WT) and 52.9 °C (R7_47D) after 45 min	Error-prone PCR is paired with directed evolution and variant library generation. When it comes to tributyrin, glyceryl error-prone PCR is combined with directed evolution and variant library creation. The R7_59A mutant outperformed the WT in terms of activity against tributyrin, glyceryl trioctanoate, coconut oil, glyceryl	Alfaro-Chávez et al., 2019

(*continued on next page*)

Table 2 (*continued*)

Name of the Enzyme	Source of the Enzyme	Name of Expression Vector	Name of Cloning vector	Molecular Mass	Optimum pH	Thermal Stability	Remarks	Reference
							trioleate, and pNP (pNP-C3, pNP-C8, pNP-C16, and pNP-C18) substrates. Using the pNP-C8 substrate, glyceryl trioleate was 228 times more active than the WT.	

unicellular form facilitates genome engineering and necessitates uncomplicated culture conditions. It carries out numerous PTM alterations that are typical of higher eukaryotes, leading to a greater level of biologically active lipases. (Cregg et al., 2000) Because *K. phaffii* has minimal nutritional requirements and may be cultivated at larger cell densities, production costs may be lowered.

Promoters are essential for establishing a cell factory that produces proteins efficiently. (Wang et al., 2013) Formaldehyde is produced from methanol via the widely used inducible alcohol oxidase 1 gene promoter. Methanol has the potential to be combustible, hence attempts are being made to find or make promoters that generate protein at least as well as or better than methanol. (Cregg et al., 2000).

Glyceraldehyde-3-phosphate dehydrogenase promoter (pGAP) is one constitutive promoter that can be utilised to make fermentation cultivations more economically viable. Lipase produced from Fusarium solani NAN103 (FSL) expressed under pAOX1 or pGAP demonstrated the highest specific lipase activity and the shortest cultivation period, two days. In *K. phaffii*, a variety of promoters with varying strengths and characteristics can be expressed; experimenting with these promoter types is a useful place to start when developing and refining the heterologous expression system. (Wongwatanapaiboon et al., 2016).

Recombinant protein expression is improved by increasing the copy number of a target gene. Increasing the copy number to three in *K. phaffii* resulted in a 2.3-fold improvement in lipase CALB production (Robert et al., 2019). Vast gene dosage, however, can hurt production because vast amounts of recombinant protein that must be appropriately folded can overwhelm the secretory system (Hohenblum et al., 2004).

To achieve its goals, the unfolded protein response (UPR) decreases protein translation, breaks down the proteins which are misfolded, and increases molecular chaperones synthesis that aid in folding of proteins. It has been shown that overexpressing and co-overexpressing chaperones in *K. phaffii* increase protein secretion and production yield. The synthesis of lipase has also risen due to the overexpression of various chaperone combinations (Jiao et al., 2018).

7. Structural characteristics of lipases

Comprehending the enzyme structure is essential for enhancing its production and utilizing site-directed mutagenesis for protein engineering. The active core domain of lipases consists of eight parallel β strands that create a central β sheet that is twisted super helical and surrounded by various numbers of α helices (Schrag et al., 1994; Gupta et al., 2015). A catalytic trio made up of an acid (Asp or Glu), a his, and a nucleophile amino acid (Ser) is the basis of the catalytic mechanism of lipases (Mala and Takeuchi, 2008). The oxyanion hole is important to stabilize the transition state in the catalysis process. It has been observed further that the catalytic triad of lipase remains covered with the help of two mobile peptides known as lid that helps in controlling the substrate channel to the catalytic center. It possesses conformational allostery with the help of interfacial activation that on contrary regulates the catalytic function and dynamics (Chen et al., 2022).

8. Protein (Lipase) engineering

Protein engineering is a process to modify the sequences of primary amino acid of new enzymes to make a better version of them. The technique has produced outstanding results in the development of optimised lipases, which are now used in a variety of industrial applications. Techniques like directed evolution and rational design have shown intriguing improvements in several lipases (Fig. 3). At present times hybrid machine learning experimental approaches can be used for determining the production of lipases (Sarmah et al., 2022). At present the use of artificial intelligence (AI) has been an important method in the enzyme engineering. Models on protocol based optimised network is used for the purpose to predict the production of lipases along with the properties such as stability, specificity of substrate and catalytic activity. Various types of network models also help in predicting and reforming of the lipase. Various networks and algorithm helps in predicting the maximum yield of the lipase (Ge et al., 2023). In order to purposefully create novel biocatalysts, rational design makes use of extensive computational modelling techniques and prior understanding of protein structure (Tiwari et al., 2012). The Protein Data Bank (PDB) has a large number of lipase structures deposited in it, and various databases provide information on lipase sequences, which has greatly aided in the rational design of these proteins. A wide range of modelling software has been generated, which facilitates the use of this methodology and increases the modelling forecast success rate.

By offering atomistic details on the dynamic molecular interactions that control protein stability and function, molecular dynamics (MD) simulations have advanced in this approach (Childers et al., 2017). In order to identify the best enzyme variations, directed evolution requires time-consuming and high-throughput screening. However, the result is libraries containing random mutations in the target gene. By combining the first two strategies and using the saturation mutagenesis technique to create libraries of mutant genes, semi-rational design is created (Sayous et al., 2020; Tripathi et al., 2014).

The number of modifications required to convert an unappealing lipase to an enzyme which has improved characteristics and potential for usage in industrial applications. With eight sequence alterations compared to the wild-type lipase, the best variation, R7_59A, displayed an activity that was 4, 5, 6, and 12 times higher than the wild-type lipase towards substrates such as glyceryl trioctanoate, tributyrin, coconut oil, and glyceryl trioleate, respectively (Alfaro-Chávez et al., 2019).

9. How omics is used to prospect for lipases?

The phenotype of an organism is determined by biological systems using the information transfer paradigm of DNA, RNA, and proteins. For many years, scientists have used genomics, transcriptomics, and proteomics to examine these “omes.” Furthermore, recent applications of metabolomics and epigenomics have been made to address queries about the many roles that organisms play. With “omics” technology advancing year after year, there is a tremendous expansion in the amount of data that can be collected in individual investigations. In addition, the present high throughput nature of these process has lowered the cost and time associated with accessing this information. This

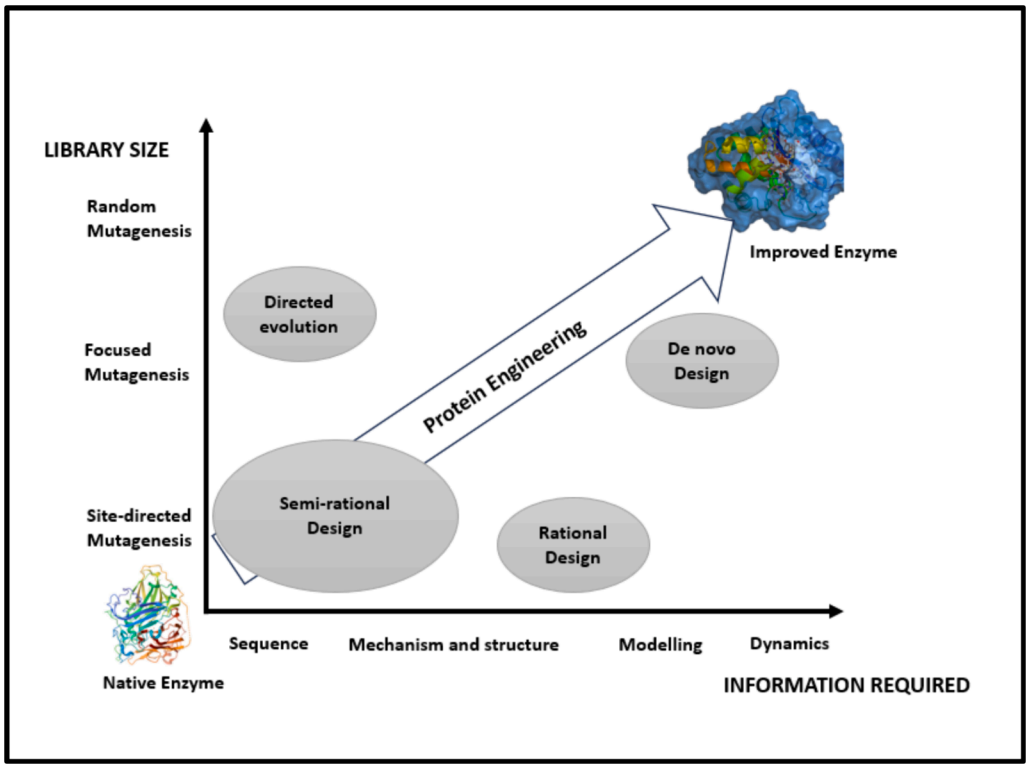

Fig. 3. Protein Engineering strategy.

has made it possible for numerous researchers to gather multiple omics data sets from a particular experimental sample. For making more thorough inferences about biological processes, various data sets need to be combined and examined as a whole.

A multi-omics approach's technologies have many things in common, some of which are different from the molecular biologists' initial plan. Until now, molecular biology has mainly relied on reductionist methods. Using this process, a large problem is divided into smaller components, each of which is solved separately. Reductionism has had considerable success, especially when the experimental subject can be explained by interactions between single molecules or is regulated by a single component. However, reductionism has important drawbacks as well.

When parts of a complex system are isolated, their function within the system is frequently lost, leading to these restrictions. When compared to a reductionist approach, this is the single biggest advantage that omics technology can offer. By keeping these elements in place, observations can be made in a real-world setting where emergent characteristics can also be investigated. Comparing these high throughput, top-down methods to the reductionist approach, they likewise yield an incredible amount of data.

Omics technologies involve thorough screening of metagenomic libraries which is a collection of clones with various DNA fragments that were produced from varied environmental samples. Both functional screening and DNA sequencing of the cloned fragments can be used to search this metagenomic library for genes of interest. Functional screening looks for clone generation, produces interesting compounds like biosurfactants, enzymes and antibiotics.

The genetic variety of the sample collected, the effectiveness of the screening procedures, and the cloning techniques (vectors and expression systems utilized) are all important factors in the successful finding of Diglyceride oil-producing lipase using metagenomics (Xing et al., 2012). Finding an economically effective high-throughput screening approach for the desired function is not always easy, because metagenomic libraries generally contain thousands of DNA sequences, making it difficult to screen for genes of interest (Choi et al., 2014).

The benefits and drawbacks of these two screening techniques—functional screening and sequence-based screening—are discussed in the subsections that follow.

10. Functional Screening

The most popular screening technique in metagenomic investigations looking for lipases is functional screening because it relies on detecting the required activity rather than requiring knowledge of the gene's sequence beforehand. The mechanism of functional screening is a sequence homology screening that helps in the identification of enzymes on the basis of their activity. It also helps in the identification of genes responsible for coding of the enzymes (Reyes-Duarte et al., 2012). The drawback of this approach is that, depending on the heterologous organism employed for expression, gene expression might not happen (Madhavan et al., 2017). Due to the incompatibility with the heterologous expression system, even in the unlikely event that gene expression occurs, expression levels may be modest (Choi et al., 2014). The primary causes of these expression incompatibilities include challenges in identifying the promoter, ineffective protein translation, and the absence of post-translational modifications required to produce active proteins (Uchiyama et al., 2005). Furthermore, the active co-expression of the lipase-specific foldase (Lif) is necessary for many lipases, yet the foldase sequence is absent from many sequences acquired through metagenomics.

11. Sequence-based screening

DNA fragments can be gathered from the nature and sequenced immediately via sequence-based screening, or they can be cloned into a heterologous host to build a library before being sequenced. To identify motifs and domains that are commonly conserved in the target enzyme pattern, the generated sequence bank is examined. The flanking portions of these conserved sections are amplified by PCR to yield the whole gene

after they have been identified. Sequence-based screening eliminates this drawback of functional screening because it does not essential that the cloned genes be expressed in a heterologous host first. The advent of robust high throughput DNA sequencing platforms made sequence-based screening techniques possible, resulting in the creation of numerous databases that contain metagenomic sequences and the identification of numerous genes that may potentially code for functional proteins within these databases.

Bioinformatic techniques are utilized to scan metagenomic sequences for determination of conserved domains or motifs of lipases and esterases, specifically in the case of lipases. The sequences that served as the basis for these conserved domains have already been deposited in databases, including Pfam, NCBI, CAZy (the database of carbohydrates-active enzymes), and UniProt (the Universal Protein Resource) (Peña-García et al., 2016). A list of esterase genes along with putative lipase is produced as a result. The mechanism of sequence-based approach helps in the retrieval of functional lipases from the microbial databases. This mechanism helped in identification of five lipolytic proteins from organisms *Agrobacterium tumefaciens, Archaeoglobus fulgidus* and *Deinococcus radiodurans* (Kwoun et al. 2004). Sequence-based screening is not without its limitations, though. Due to the method's reliance on homology with known sequences, sequence-based screening has the drawback of only being able to identify novel variations of functional classes of enzymes (Xiao et al., 2015; Simon et al., 2011). Further reducing the possibility of discovering new enzymes is the fact that DNA obtained from the most common organisms is more well-represented in metagenomic libraries than the other rarer members of the communityIt is normal to find proteins with known amino acid sequences and high homologies (above 50 %, frequently around 80–98 %).

12. Proteomic study in lipase identification

Proteomics, like transcriptomics, can carry out qualitative and quantitative analysis of every protein expressed by lipase-producing microbes under various scenarios. Now, the most used method for lipase-producing microorganisms is comparative proteomics. Combining this data with other omics data sets makes it most useful. Even with the recent significant advancements in proteomic technology (Gillet et al., 2016), the throughput of this omics data collection is limited when combined with transcriptomics and genomics. Data Independent Acquisition (DIA), the acronym for untargeted discovery proteomics, enables the most thorough conjunction with other omics sets (Hu et al., 2016). To provide functional data, this technique makes it easier to track genes to proteins (Trapp et al., 2016, Tocchettii et al., 2015). Although the mRNA levels mentioned above are inherently linked to protein abundance, there is a poor correlation between mRNA abundance and protein abundance in a system (Chen et al., 2002; Pascal et al., 2008). Given this discrepancy and the fact that practically all biological functions are regulated by proteins, it is obvious that combining both technologies will provide a more comprehensive picture (Cox et al., 2007). When integrated, these data sets can provide comprehensive answers to higher-dimensional issues about large-scale processes, such as the holistic study of many products' simultaneous metabolism (Wang et al., 2013). This method can be used to interpret intricate microbial interactions like quorum sensing (Cagno et al., 2011). Proteomic tools allow the mechanism of high-throughput analyses, functional identification of proteome and large-scale production (Chandramouli and Qian, 2009).

13. Metagenomic study of oil production

Recently, there has been a significant use of metagenomic approaches to separate and discover new enzymes from non-cultivable portions of microbial populations. Among all these enzymes, one of them is lipase, which have been isolated and studied from a variety of environmental sources. Although a number of these lipases have properties that make them promising candidates for biocatalysis, little research has been conducted to assess their ability to catalyse critical industrial reactions.

After identifying lipase-producing clones through screening, they must be generated, purified, and characterised. Typically, the expression of a new lipase involves the following steps: Cloning lipase-coding genes into an expression vector involves identifying an active lipase expression system, sequencing the clone, analysing the gene sequence, and over-expressing the lipase. There are numerous prokaryotic and eukaryotic host cells, expression vectors, and cloning vectors to choose from.

To aid in the lipase's purification using affinity chromatography, an affinity tag is frequently added to either the N- or C-terminal of the enzyme during cloning and expression. The histidine tag is the most widely used because it enables one-step purification in a nickel column (Terpe et al., 2003). Following purification, the novel lipase is biochemically characterized using tests to measure stability, kinetic constants, and activity with different substrates. Usually, the lipase needs to be immobilized before it can be utilized in synthesis activities in organic solvents. The lipase's three-dimensional structure can also be investigated, either directly by X-ray crystallography or indirectly through homology modeling, which involves sequence comparison to databases that contain sequences of lipases with 3-D structures.

14. Metagenomic lipases: An application in biocatalysis

It has been suggested that several of the lipases discovered using the metagenomic approach had characteristics that would make them desirable for application in industrial operations. Nevertheless, the application of metagenomic lipases in product or process formulation is limited to a small number of patents. Thermomyces lanuginosus lipase, which had previously been utilized in an analogous detergent formulation (Olinski et al., 2015), exhibited activity and stability like that of lipase Lipr138, which was employed in a detergent formulation. To synthesize polylactic acid polymers, several lipases were extracted from a metagenomic library produced from samples of rumen contents (Prive et al., 2014). Furthermore, patents have been granted for the resolution of racemic substrates and the use of metagenomic lipases in biodiesel (Zhiyang et al.,).

Metagenomic lipases have not been thoroughly studied in synthesis processes. Only 5 of the 288 enzymes found in a 2016 review of metagenomic lipases and esterases had been assessed in synthesis reactions (Ferrer et al., 2016). This demonstrates that throughout the past three years, there has been a rise in interest in assessing the uses of metagenomic lipases in synthesis.

15. Conclusion

Significant advancements have been made in the mechanisms that produce omics information in recent years. The emergence of novel technologies, like third-generation sequencing, has the potential to revolutionize the amount of data at researchers' disposal. More scientists can now integrate numerous omics data sets because of these advancements. Significant progress will be made in all facets of microbial research because of this availability, from identifying roles to comprehending how they fit into intricate ecosystems. In examining organisms and systems at the same time, this review evaluates the benefits of using a high dimensional systems-level approach. Biologists must stay on top of this trend as the field of molecular biology gets more and more data intensive. In addition to being adaptable to integrating new technologies into their workflows, researchers need to have expertise in data processing and comprehend the workings of the software they use. Even though this work is difficult, multi-omics offers significant benefits.

16. SWOT analysis of the review

16.1. Strength

This review focuses on emerging and specific area of research which combines the concept of genomics and proteomics in the production of the lipase.

This review is an interdisciplinary where it not only provide the concept of genomics, metagenomics and proteomics but also mentioned the use of artificial intelligence and machine-learning in the optimization of lipase production and also finding its efficiency.

This review thus helps the reader to understand various techniques those can be used for the lipase production.

16.2. Weakness

Till now very insignificant work has been done in studying lipase and lipase producing organisms in the field of genomics and proteomics. Thus this review helps in understanding the gaps in the present day research.

Limited data availability on certain lipase producing microbe.

16.3. Opportunities

Certain microbial lipases have some properties those facilitate the production of novel lipases having properties those resemble various industrial applications.

The metagenomics studies have also explored in identifying the lipase producing organisms.

16.4. Threats

Potential ethical issue develop in various types of genetically modified species.

CRediT authorship contribution statement

Debashrita Majumder: Writing – original draft, Conceptualization. **Ankita Dey:** Writing – original draft. **Srimanta Ray:** Writing – review & editing. **Debasmita Bhattacharya:** Writing – review & editing, Conceptualization. **Moupriya Nag:** Writing – review & editing, Project administration. **Dibyajit Lahiri:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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