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# Metagenomic Lipases and their Applications in the Food Industry

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## Abstract
The metagenomic method allows researchers to capture a broader spectrum of microbial diversity compared to traditional culturing techniques. Frequently, a sustainable portion of microorganisms in the environment, which cannot be grown in a laboratory setting, are often overlooked. By directly isolating and sequencing genomic DNA from the environment with this nature, scientists can gain insights into the full extent of microbial diversity in natural habitats. Lipase enzymes work by catalyzing the hydrolysis of lipids, which are fats and oils, into their component molecules. Metagenomic lipase enzymes have a wide range of applications in the food industry, such as enhancing taste characteristics, refining texture, and prolonging the shelf life of various food products. These enzymes can be sourced from environmental samples, providing a wide range of novel and robust enzymes for applications such as cheese production, baking, and oil processing. Their ability to catalyze lipid hydrolysis makes them valuable tools for creating unique and innovative food products.

**Keywords:** Metagenomics; Biocatalysts; Function-based screening; Sequence-based; Novel application of lipases

## Introduction
Metagenomics is a potent method for examining the genetic material of all microorganisms in a specific habitat. Traditionally, microbiologists have relied on culturing techniques to study microorganisms, which limits their ability to study the vast majority of microbes that do not easily grow in laboratory conditions. Metagenomics allows scientists to directly retrieve and analyze the genetic material from a community of microorganisms, offering valuable insights into the variety and roles of these microbial residents. This includes bacteria, archaea, viruses, fungi, and other microorganisms. The genetic material can be extracted from an environmental sample, like soil, water, or gut microbiomes (Sabree et al., 2009). Metagenomics has brought about a profound transformation in our comprehension of microbial communities and their possible uses across various domains. It enables us to explore the genetic diversity of these communities, uncovering new species and their functional capabilities. This has far-reaching implications for biotechnology, as it opens doors to discovering novel biocatalysts and other valuable resources. The data from extreme environments, in particular, hold promise for unique and resilient biotechnological solutions (Lorenz et al., 2005). It involves the retrieval of DNA from environmental samples, which is then preserved in libraries using different host organisms.

In this regard, researchers can search for metagenomic libraries for specific genes through sequence-based methods or express the DNA to identify desired enzymes with specific activities (Selvin et al., 2012).

Metagenomic methods are utilized like amplicon sequencing to characterize the microbial community composition. Analyzing microbial communities also reveals the functional roles played by different microorganisms in a community, including the identification of undiscovered enzymes that can be applied in biotechnology and the fields of biosciences. This interdisciplinary approach holds great promise for comprehending and utilizing microbial diversity for various purposes (Lorenz et al., 2005). Its application includes the ability to selectively act on specific chiral and regioselective sites has harnessed chiral drug resolution, modifying fats, producing cocoa butter substitutes, biofuel synthesis, and enhancing flavors. Lipase-catalyzed processes have garnered substantial interest due to several advantages, including their environmental friendliness compared to bulk chemical syntheses, capacity for producing higher-quality products, ease of recovery and reuse, and suitability for continuous operations (Khan et al., 2013). The metagenomics era brought significant progress by studying the metagenome, which encompasses genomes in microbial communities. Advanced molecular techniques enable the creation of environmental DNA libraries, revealing genomes of previously unculturable organisms. This opens opportunities for discovering novel enzymes with unique properties, many of industrial relevance. Metagenomic studies are anticipated to uncover more enzymes of interest than traditional methods, with a particular focus on specific groups such as lipases and esters (Lopez et al., 2014). Various methods are used in functional metagenomics to discover novel enzymes useful in human society. These techniques encompass gene construction, screening, gene expression, and subsequent bioinformatics assessments. Additionally, the characterization of protein products involves determining factors like optimal pH and temperature rates, as well as analyzing protein activity (Prayoga et al., 2020). Metagenomic techniques are essential for identifying are essential all the genes in a given community, using both functional and sequence-based screening methods. The dominance of shotgun sequencing has been replaced by high throughput third-generation sequencing (TGS) and next-generation sequencing (NGS) technologies, which offer the advantage of rapidly identifying pathogenic microorganisms (Gokilavani et al., 2023).

***Overview on lipases***

Lipases are highly adaptable enzymes with a broad spectrum of functions, and they are essential in various reactions. These applications highlight the significant impact of lipases in food processing and product development (Khan and Sathya, 2018). Many microbes have been found to produce lipases, but only a limited subset of these microbial lipases has been extensively studied, purified, and utilized in industrial settings (Verma et al., 2021). This approach likely facilitated the efficient discovery and study of these enzymes from various sources (Amani et al., 2022). The diverse applications of lipases span multiple industries, encompassing formulation, disinfectant synthesis, pharmaceuticals, paper manufacturing, nutrition, cosmetics, and pharmaceutical processing. Their versatile enzymatic properties make them valuable tools in various life sector fields Jeon et al. (2011). Lipases, specifically triacylglycerol hydrolases are indeed important enzymes in various industrial food processes (Khan et al., 2020).

***Research in metagenomic lipases***

Researchers are currently in competition to investigate alternative organisms with the potential to enhance lipase production (Liu et al., 2007). In this regard, they are currently utilizing metagenomic methods to identify new, which show promising effects for industrial use (Lopezo et al., 2014).

The process of PCR amplification involves using metagenomic DNA extracted from a fosmid within a metagenomic library. A fosmid in a metagenomic library is a common technique used in microbiology and genomics. PCR amplification is a powerful tool for studying specific genetic elements within complex metagenomic samples, allowing researchers to investigate the genetic diversity and functional potential of microbial communities in environmental samples. While

PCR itself may not be used to amplify lipase enzymes directly in food production, it can be employed in the development and quality control of enzyme-producing microorganisms. Lipase enzymes can be produced by genetically engineered microorganisms.

***Isolation of 16s rRNA***

Metagenomic clone libraries are incredibly valuable tools. This approach allows researchers to bypass the limitations of cultivation and PCR-based techniques, providing insights into previously unexplored aspects of microbial metabolism and phylogeny. (Cottrel et al., 2005). It is extensively utilized as a tool for the phylogenetic identification of bacterial strains found in environment samples (Kevin et al., 2019). The choice of genes to amplify is a pivotal stage in metagenomics research, and in numerous investigations, employed to examine the diversity within complex microbial populations (Kamble et al., 2020).

***Function-based Screening***

Plating clones on agar plates is a common method for screening metagenomic libraries to identify enzymes like lipases. Different substrates are used to select for specific activities. For instance, (Su et al., 2015) screened a sea sponge microbiota library and isolated a from 6568 clones. Screened a hot spring water sample library and found 88,000 clones. (Zheng et al., 2015). screened an oil-contaminated soil library identifying and obtaining used a three-stage screening approach with different substrates, resulting in the isolation library containing clones contaminated with animal fat. This method helps discover enzymes with specific activities from diverse environmental samples.

***Sequence-based Screening***

Metagenomic library screening showed that LipC12 could be valuable for applications in the food industry. Caprylic acid, a medium-chain fatty acid, has various culinary and functional uses in food products due to its unique properties (Madalozzo et al., 2016). The process of expressing and characterizing the metagenomic lipase LipC12 has been reviewed and formulated in various food processing industries. This comprehensive approach involves cloning, transformation, purification, characterization, and structural analysis. The challenges with lipases that require specific enzymatic reactions are also highlighted, along with these strategies to address them to the next level (Almeida et al., 2019).

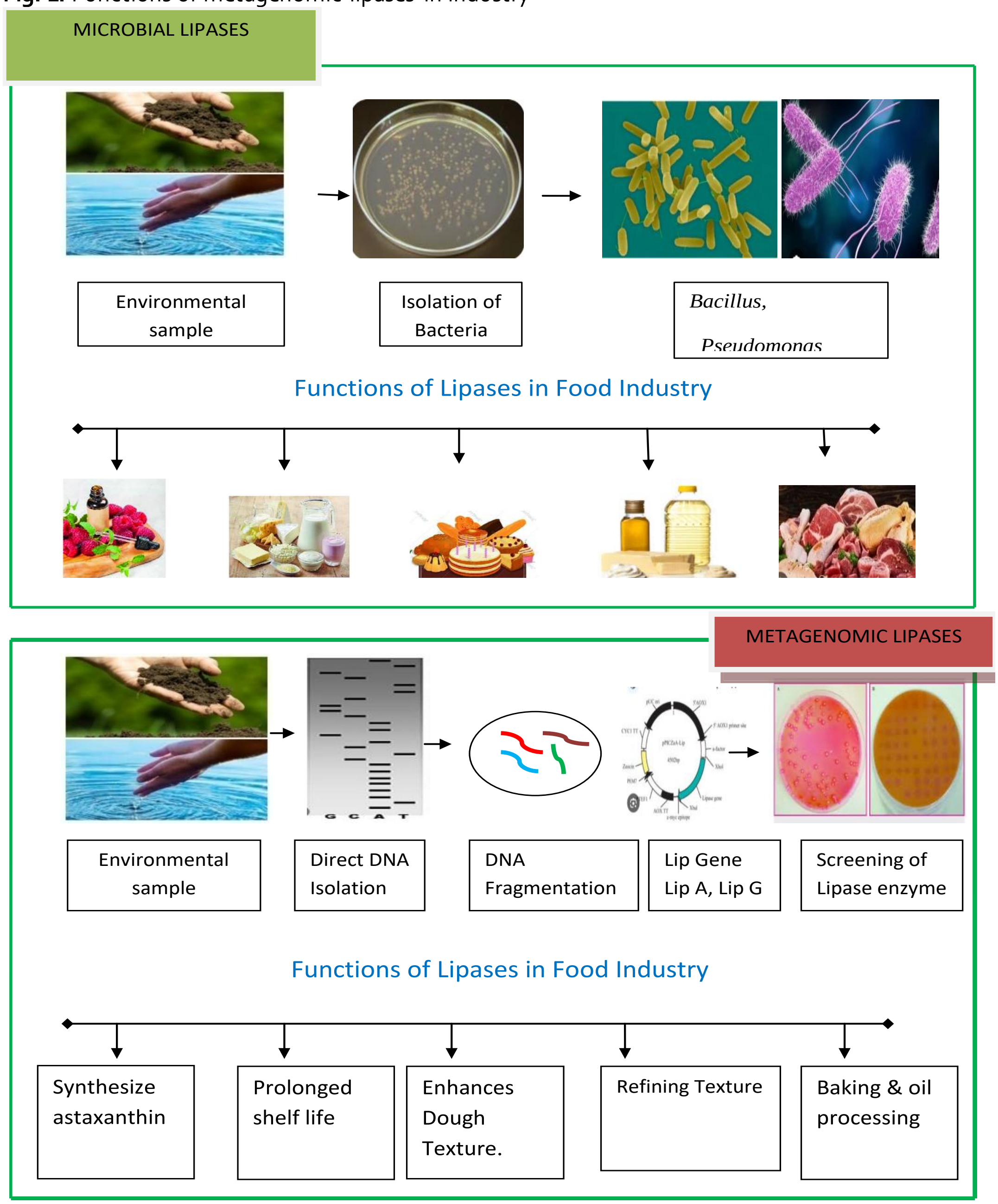
**Applications**

***Metagenomic lipase in the food industry***

Metagenomic lipase enzymes have a wide range of applications in the food industry, such as enhancing taste characteristics, refining texture, and prolonging the shelf life of various food products. These enzymes can be sourced from environmental samples, providing a wide range of novel and robust enzymes for applications such as cheese production, baking, and oil processing. Their ability to catalyze lipid hydrolysis makes them valuable tools for creating unique and innovative food products. Lipases are employed in degumming vegetable oils, modifying fats to yield higher-value items with elevated polyunsaturated fatty acid content. (Chandra et al., 2020). They can improve the texture and softness of cheese by breaking down fats, enhancing the flavor of butter and margarine, altering the aroma, and elevating the quality of cocoa butter (Raveendran et al., 2018).

Lipase, which can form during vegetable oil processing, often contains impurities like phospholipids. Recombinant lipase enzymes can be effective in degumming vegetable oils by breaking down these phospholipids, making the oil suitable for various applications. These enzymes, often produced through genetic engineering techniques, can enhance the efficiency of degumming processes by being employed to address the issue of acidification in lakes caused by the accumulation of food waste (Liu et al., 2007). Lipase in the process of transesterification can be considered for converting free fatty acids into methyl esters in the context of addressing acid lakes. (Adina et al., 2021). Antarctic metagenomic lipases are enzymes derived from microorganisms found in extreme cold environments, such as the Antarctic. These lipases have unique properties that make them valuable in various biotechnological applications, including their use as biocatalysts in non-aqueous (organic solvent) environments. The use of Antarctic metagenomic lipases in non-aqueous biocatalysis offers several advantages. These lipases are known for their stability and activity at low temperatures, which can be advantageous in non-aqueous reactions where maintaining low temperatures may be challenging. Additionally, they often exhibit a broad substrate specificity, where enzymatic reactions in non-aqueous environments can be more efficient and environmentally friendly compared to traditional chemical processes. Their enzymatic action can often lead to more precise and controlled reactions, which is beneficial in the food industry for achieving consistent product quality (Su et al., 2006).

**Fig. 1.** Functions of metagenomic lipases in industry




Salt pans lipase has a role in the food industry as a source of salt, essential for food preservation and flavor enhancement. However, they are not directly connected to metagenomics. Salt pans lipase can be used to identify lipase-producing strains, which can then be employed to improve the flavor of fish sauce during its production process (Boopan et al., 2007). Salt pans can provide a valuable resource for uncovering the fermentation process involving lipase-producing microorganisms (Esterbantorress et al., 2015). Utilizing soda lake lipase in the food industry can enhance fish oil by increasing its polyunsaturated fatty acid content, which is commonly used in food products (Coa et al., 2020). Soda lake lipase is an enzyme that can be used in the food industry to help synthesize astaxanthin. Astaxanthin is a red pigment and a powerful antioxidant commonly found in seafood such as salmon and shrimp.

**TABLE 1.** Sources and Functions of Metagenomic Lipases in Food industry

| Source | Function /Application | Reference |
|---|---|---|
| Marine water | 1. Lipolyzed milk fat is created by breaking down milk fats using enzymes to enhance flavor in food<br>2. Texture enhancement in the dairy industry.<br>3. Lipolysis level affects richness and cheesiness in dairy products.<br>4. Non aqueous biocatalyst | Hasan et al. (2006)<br>Qing et al. (2014)<br>Vivek et al. (2022)<br>Su et al. (2006) |
| Marine sediment | 1. The enzyme displays a strong preference for esters found in buttermilk fat.<br>2. This enzyme generates distinctive flavors by producing palmitic and myristic acids when interacting with butter.<br>3. The enzyme preserves the cheesy taste. | Prayogo et al. (2020)<br>Sharma et al. (2001)<br>Hasan et al. (2006) |
| Hot spring | 1. Potential biocatalyst | Vivek et al. (2022) |
| Deep sea sediment | 1. The enzyme is utilized in dairy and food industries and waste oil treatment. | Vivek et al. (2022) |
| Environmental sample | 1. Texture enhancement in the dairy industry.<br>2. Lipolysis level affects richness and cheesiness in dairy products.<br>3. Cheddar cheese development.<br>4. Enhancing cheese maturation.<br>5. Utilizing food technology in the fats and oils, dairy, and bakery sectors.<br>6. Degradation of industrial pollution viz., Cereal wastes, plastics, and other toxic chemicals.<br>7. Lipases, as versatile biological catalysts, offer a promising outlook for fulfilling the requirements of several industries.<br>8. Furthermore, lipases assist in eliminating fat.<br>9. Cheese flavor development | Vivek et al. (2020)<br>Raveendran et al. (2018)<br>Jooyandeshet al. (2009)<br>Araveindan et al. (2007)<br>Law et al. (2009)<br>Pandra et al. (2005)<br>Chandra et al. (2020)<br>Sharma et al. (2014)<br>Joojendeh et al. (2009) |
| Agricultural soil | 1. The utilization of lipases sourced from microorganisms finds application in diverse sectors, including the dairy and food industries. | Ray et al. (2012) |
| Ground Soil | 1. Enhancing the aroma in beverages is achieved through cheese ripening and the hydrolysis of milk fat.<br>2. The quality of edible oils and fats can be upgraded for various culinary uses.<br>3. In situ emulsification enhances dough texture. | Vivek et al. (2022)<br>Jooyandeh et al. (2009) |
| Salt pans | 1. Fish oil can be processed to produce polyunsaturated fatty acids, such as omega-3s, through a method called transesterification.<br>2. The flavor enhancement of dishes can be achieved by fermenting fish with salt to create fish sauce.<br>3. Fermentation process. | Perez et al. (2011)<br>Kanalytakrit et al. (2007)<br>EsterbanTorress et al. (2015) |
| Soda lake | 1. Astaxanthin synthesis | Cao et al. (2020) |
| Acid lake | 1. The use of recombinant lipase has proven to be efficient for the removal of gum impurities in vegetable oil.<br>2. Employing bioremediation methods for the treatment of food waste presents a sustainable solution.<br>3. Transesterification, which converts free fatty acids into methyl esters. | Prez et al. (2011)<br>Kanaltakrit et al. (2007)<br>EsterbanTorress et al. (2015) |

***Lipase in the dairy industry***

Lipolyzed milk fat is generated through the enzymatic breakdown of milk fats to improve flavor in food, while it also serves as a texture enhancer in the dairy sector (Qing et al., 2014).

Moreover, the degree of lipolysis plays a role in determining the richness and cheesiness of dairy products (Vivek et al., 2022). The enzyme used in this context demonstrates a remarkable specificity when interacting with the esters found in buttermilk fat. Its action leads to the formation of palmitic and myristic acids, which in turn contributes to the development of a unique flavor profile, particularly when incorporated into butter. Furthermore, this enzyme plays a pivotal role in preserving and enhancing the cheesy flavor characteristic of the product in question (Prayogo et al., 2020, Rohit Sharma et al., 2001). Hot springs are natural geothermal features that can serve as potential biocatalyst environments due to their high temperatures and unique mineral compositions. Researchers often explore hot springs as a source of novel biocatalysts for their stability and efficiency under extreme conditions (Vivek et al., 2020).

***Lipases in waste treatment***

Deep sea sediment and then transitioned to talking about enzymes used in dairy, food industries, and waste oil treatment. In the realm of the dairy industry, there is a significant focus on texture enhancement. The richness and cheesiness of dairy products are directly influenced by the level of lipolysis (Vivek et al., 2020) Cheddar cheese, a beloved dairy product, undergoes a unique development process (Raveendran et al., 2018). Furthermore, there is a notable interest in accelerating the ripening of cheese and promoting lipolysis in various sources, be it milk, vegetable oil, or fat, to attain specific and distinct flavors. These principles extend beyond dairy, to food technology (Aravindan et al., 2007).

***Lipases in the protection of the environment***

Efforts to mitigate the detrimental effects of industrial pollution, encompassing issues such as cereal waste, plastics, and harmful chemicals, have become increasingly important. Lipases, recognized as multipurpose biological catalysts, present a favorable outlook for various industries, particularly in the realm of food and beverages (Chandra et al., 2020)Their unique enzymatic properties offer promising solutions, from enhancing food processing, contributing to improved product quality and sustainability (Sharma et al., 2014) Pancreatic enzymes, like lipases, are indeed used in various applications for their ability to break down fats and improve processes in the food industry, including cheese production and oil processing (Ray et al., 2012).

These processes play crucial roles in various aspects of food production and culinary arts. Firstly, cheese ripening and the hydrolysis of milk fat are essential techniques for improving the aroma of beverages. They contribute to enhancing the overall flavor profile, making beverages more enjoyable. Secondly, there is a strong emphasis on improving the quality of edible oils and fats. This quality enhancement ensures that the oils and fats used in cooking and food preparation meet the highest standards, thereby elevating the taste and nutritional value of the final dishes. Lastly, in situ emulsification proves to be a valuable tool in the culinary world as it enhances dough texture. This process contributes to the creation of perfect dough, leading to the production of delightful bread and pastry products. In summary, these processes are vital for optimizing various aspects of food and beverage production, ultimately leading to more enjoyable and delicious culinary experiences (Vivek et al., 2020).

## Conclusion

Metagenomic lipase enzymes have proven to be invaluable tools in the food industry. Their ability to catalyze lipid hydrolysis reactions offers numerous advantages, including enhanced flavor development, texture improvement, and extended shelf life of various food products. Moreover, their sourcing from diverse microbial communities via metagenomics has expanded the pool of available enzymes, increasing the potential for tailored applications. As the food industry continues to seek sustainable and efficient solutions, metagenomic lipase enzymes are likely to play an increasingly prominent role in meeting these demands.

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PSG, SV, KG, TAS and ABK conceived the concept, wrote and approved the manuscript.

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