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LIPASE-PRODUCING BACTERIA AS PROBIOTICS AND RECENT ADVANCEMENT

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Abstract:
Microbial lipases, in particular, are becoming more valuable because they can speed up a wide range of chemical reactions in both water and dry environments. The global market for lipase is projected to reach USD 797.7 million by 2025, growing at a compound annual growth rate (CAGR) of 6.2% between 2017 and 2025. The creation of novel and improved lipases using molecular techniques is a recent development in the field of lipase research. As an illustration, the merger of controlled enzyme evolution and rational enzyme design to achieve desired features in lipases. As they hydrolysed fats into fatty acids and glycerol at the water—lipid interface and may reverse the reaction in non-aqueous environments, lipases stand out among biocatalysts and have a wide range of biotechnological uses. These enzymes' remarkable stability in organic solvents has propelled them to the forefront of organic synthesis, where they are being used in the creation of cutting-edge pharmaceuticals, surfactants, bioactive molecules, and oleochemicals. Lipase-catalysed trans- and inter-esterification reactions have also been utilized in the fat industry. Given the breadth of lipase's potential uses, the industrialization of lipase production has been a hot topic amongst microbiologists, process engineers, and biochemists. Microbes, particularly fungi, and bacteria, have been shown to be the preferred production tools in this field of research. Several microbial lipases have had their structures determined recently, expanding our understanding of the enzyme's unusual catalytic mechanism. An overview of lipase-producing bacteria as probiotics, their therapeutic applications, and their immunomodulatory properties is attempted in this paper.

Keywords: Biocatalyst, Microbial Lipase, Therapeutic applications, Modulators of lipase activity

1. Introduction:

1.1 Biocatalysis:

The concepts of "green chemistry" [1,2] reflect the fact that modern chemistry necessitates the creation of very complex products via methods resulting in significant conversions and little by-products. Enzymatic biocatalysis is proving to be an increasingly viable option in this challenging setting [3-5]; In fact, the remarkable selectivity and specificity of enzymes allows for a dramatic reduction in both substrate purity requirements and the production of by-products when they are allowed to function under mild conditions [6,7]. There are numerous industrial applications for enzymes as biocatalysts, including the food industry, energy generation, fine chemistry, polymer research, etc [8-11].

The features of enzymes are nearly optimal, yet they are only good enough for their physiological role [12]. Enzymes in living organisms need to be relatively stable but reactive, as they are constantly

exposed to a variety of substances and must respond rapidly to stress [13,14]. For instance, in kinetically regulated synthesis [15][16], an acyl donor is transferred to a different nucleophile than water [17,18] via hydrolase. Another case involves promiscuous enzymes, which catalyze reactions that are completely unrelated to their biological function (such as utilizing a hydrolase to catalyze the synthesis of carbon-carbon bonds or oxidation). Flirtatious activity is often produced in a compartment of the enzyme that is distinct from the active center [19]. As an added complication, it is well known that a reaction by-product may inactivate some enzymes. While this harmful sub-product is often removed in vivo by other enzymes, accumulation of this substance within a reactor with pure enzymes might constitute a significant problem [20]. While other enzymes in the metabolic machinery recycle these cofactors in vivo, doing so in the reactor calls for a number of complex methods [21]. Therefore, enzymes typically need to be enhanced before they can be employed in industries [22][23].

Therefore, metagenomic approaches have provided access to all biodiversity, including microbes that cannot be cultured or that no longer exist [24-28]. The cost of these biocatalysts has been considerably decreased thanks to cloning and overexpression [29]. Site-directed mutagenesis and dynamic simulations are expanding the possibilities for enzyme improvement every day [30-34], but directed evolution allows scientists to imitate natural selection in a short amount of time while focusing solely on the property of the enzyme they seek to optimize [35-37]. Altering the active site through mutagenesis (for instance, by exchanging a Ser residue for a metal) can lead to novel enzyme activity [38]. Enzymes containing two active centers can be synthesized by forming new active centers from scratch [39]. Likewise, chemical modification is constantly refining its methods, allowing for greater process control [40-42]. It has also paved the way for the creation of synthetic enzymes with novel catalytic properties. Since enzymes were first quite costly, immobilization was developed as a solution to the difficulties of enzyme reuse [43]. Although enzyme costs have decreased in some cases recently, immobilization remains an important step in biocatalyst design. Indeed, the enzyme's reliability, efficiency, preference, purity, and reduction in inhibition can all be improved with careful immobilization. [44-48]. Smart immobilization techniques can even help with the issue of cofactor recycling and reuse [49,50]. Finally, new reactor layouts may allow for more varied enzyme applications in the industry.

1.2 Lipase:

The enzyme lipase is one of the most popular ones. Because of their exceptional qualities, they are responsible for this [51,52]. The first is that they are independent of cofactors [53-55]. Second, they are extremely stable and active in a broad variety of media, including aqueous, anhydrous, and neo-media [56-59]. More so, they recognize a wide variety of substrates while having a low specificity [60], although they often do so with maintaining a high degree of regio/enantio selectivity. The pharmaceutical and fine chemical industries, the energy sector (biodiesel production), the food industry, the plastics industry, etc. [59,61,62] all rely on them. Lipases are known to function as surface enzymes [63–65]. In a homogeneous medium, the two lipase forms (closed and open) exist in equilibrium due to a movable polypeptide chain called the lid. [64,66,67]

The active center of the lipase is shielded from the environment by the lid in its closed form; for some lipases, some lipases, like the one from Bacillus stearothermophilus, have a double lid, while others, like the one from Candida antarctica, have a single lid that is too narrow to completely prevent the enzyme's action [68,69]. [70]. The active center of the lipase is exposed to the solvent in its open state, allowing the lipase's vast hydrophobic pocket to be exposed to the medium [70,71]. Lipase can digest intractable substrates after becoming strongly adsorbed to a drop of the substrate that has been treated with hydrophobic conditions [72–76].

Lipases use this adsorption technique to affix themselves to hydrophobic surfaces, such as those present on hydrophobic proteins [77-86]. As a result, immobilizing lipases onto hydrophobic carriers provides a straightforward method for achieving simultaneous immobilization, purification, stability, and hyperactivation for the vast majority of lipases. This mechanism of action makes lipases structurally very malleable [87- 89]. Therefore, they are amenable to manipulation via variation in

experimental settings, genetic engineering, chemical engineering, and immobilization methods [90]. It has been shown that, depending on the immobilization approach used, some immobilized lipases exhibit enantioselectivity in the opposite direction.

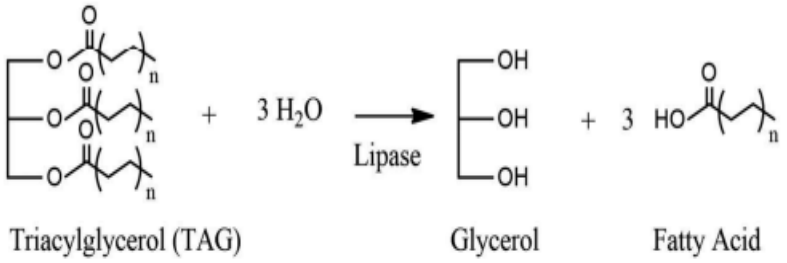


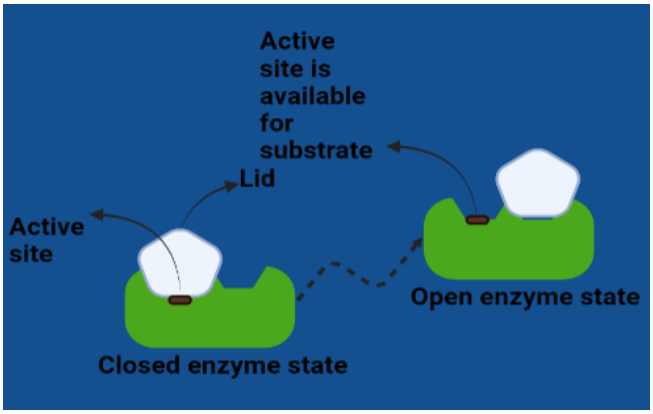
Figure 1: Overview of the Lipase Reaction [91]

2. Historical background:

As proteins, enzymes can function both within and outside of cells to catalyze a wide range of chemical and biological events. They are highly selective natural catalysts for their target substrates, resulting in high conversion rates. And they do this in places where they have limited control over things like temperature, pressure, and pH. Claude Bernard discovered lipase, an enzyme that hydrolyzes oil droplets into soluble molecules, in pancreatic juice in 1856. Bacillus prodigiosus, Bacillus pyocyaneus, and Bacillus fuorescens were later discovered to produce lipase in 1901; nowadays, large-scale lipase production has been reported in Serratia marcescens, Pseudomonas aeruginosa, and Pseudomonas fuorescens. Lipolase, the first recombinant lipase to be mass-produced for commercial use, was originally expressed in Aspergillus oryzae in 1994. Lipase, originally isolated from the pancreas of animals, has been used as a digestive aid for centuries. As a biocatalytic process, it's been put to use in the synthesis of numerous untried chemicals.[92]

3. Brief review of lipase structure and catalytic reactions:

To effectively personalize lipases, it is desirable to have access to their three-dimensional structure and the parameters affecting region and enantiospecificity. Enzymes that hydrolyze triacylglycerol acyl-esters, often known as lipases, have the EC number 3.1.1.3 and are common in nature. Despite the fact that lipases have little in common in terms of amino acid sequence, they all possess highly similar folds (- hydrolase folds). The core of these strands is extremely coiled, and surrounding it are varied -helices. Figure 2 shows that in the closed conformation, the active site is shielded from substrate moieties by a helical segment known as a lid. Interfacial activation, as depicted in Figure 2 [93], occurs as soon as lipid molecules are present, and involves the rapid movement of the lid to expose the active region. In lipases, the active site is typically represented by the sequence GX1SX2G, where G stands for glycine, S for serine, X1 for histidine, and X2 for aspartate or glutamate [94].

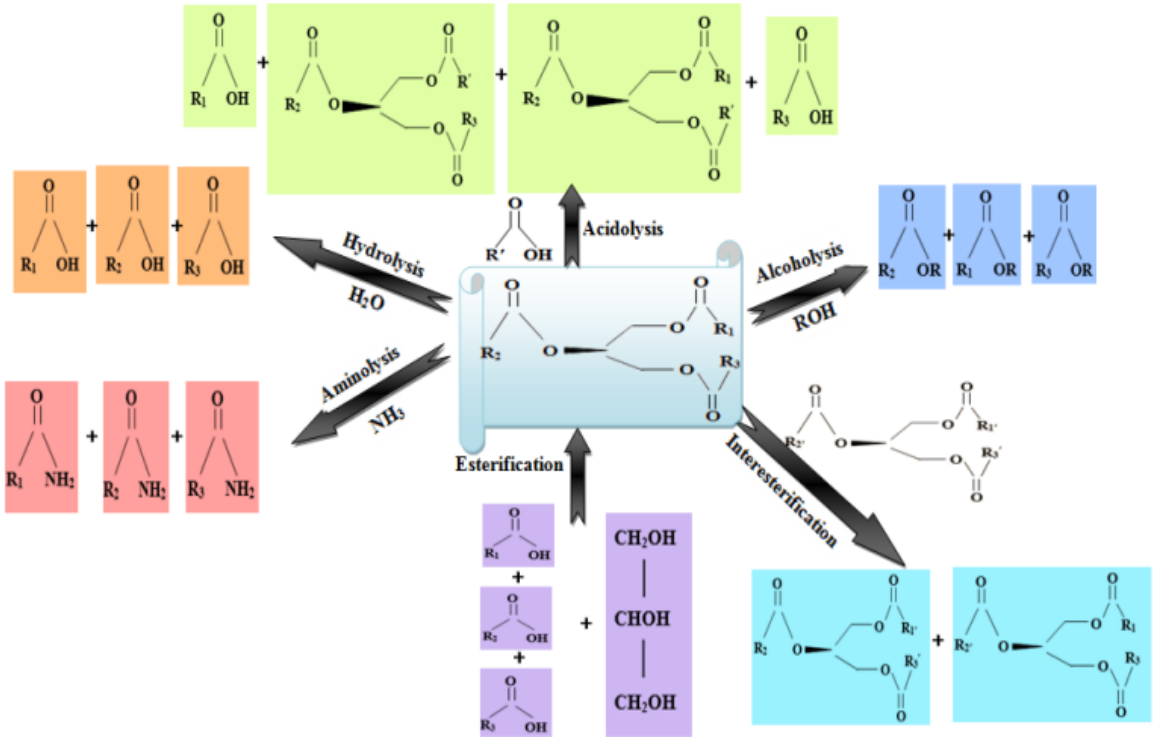


As depicted in Figure 2, lipase is activated at the interface. [101]

Adhesive chambers, lids, oxy-anion holes, and disulfide bridges are all examples of functional modules found in lipases. The binding pocket of a lipase is an elevated region of the protein, located above the core -sheet, that resembles a cleft next to the tunnel-like binding segments. The catalytic effectiveness of lipases is also affected by the oxy-anion hole, which is an essential component [95]. The lid's hydrophilic exterior faces the substance being activated, while the lid's hydrophobic interior faces away from the active region. When in an aqueous solution, the lid is closed and protects the lipase active site. Lipases undergo interfacial activation, a configurational change that puts them in an active state by dislodging the lid, when a lipid-water interface is present. Lipases are distinct from other types of esterase because they are the only ones capable of hydrolyzing water-insoluble esters [96]. Lipase from the arctic sea microflora has been shown to be more effective in some recent studies [97], a thermotolerant lipase has been isolated from Candida antarctica [98], and recombinant lipases have been produced by Bacillus licheniformis that are resistant to heat and alkali [99], among other conditions. Lipase-catalyzed reactions can be further categorized into two broad categories: synthesis reactions and hydrolysis reactions. Esterification, aminolysis, inter-esterification, alcoholysis, and acidolysis are all subcategories of synthesis reactions [100]. Some examples are provided down below.

3.1 Hydrolysis:

Hydrolysis of lipids, or lipolysis, is depicted in Figure 3; this process converts lipid or ester molecules to smaller fragments such as fatty acids and glycerol.



Lipase-catalyzed esterification, hydrolysis, aminolysis, and transesterification (Figure 3). [102]

Both aqueous and organic mediums can be used for hydrolytic processes mediated by lipase [103,104]. Several commercial products are synthesized using processes similar to lipolysis. For instance, lipase is used as a hydrolytic biocatalyst in the development of newborn milk formulas [105,106]. In order for lipase regeneration to be beneficial, many of these applications must employ enzyme immobilization techniques [107,108]. Lipases can be used in a wide variety of biocatalytic processes. Hydrolysis of lipids, esterification, aminolysis, and trans-esterification of esters are just few of the chemical reactions that lipases catalyze.

3.2 Esterification:

Two esters and one water molecule are produced in the esterification reaction between an alcohol or glycerol and a fatty acid. The esterification process can be used to remove water using a number of cutting-edge methods [109]. The introduction of a vacuum via vacuum pump is a more efficient and convenient approach for getting rid of water than the previously stated processes. Notable cases include the use of lipase isolated from Candida antarctica to catalyze the esterification of lactic acid or carboxylic acid with an alcohol. Esterification of dihydroxystearic acid using lipases isolated from Rhizomucor meihei and Candida antarctica, [110], etc. For the manufacture of ester from used cooking oil, S. aureus lipase MNPs has also been used [111]. Esterification processes mediated by lipases are widely used in the food industry, especially for generating glycerides from fats and oils. Sugar alcohols and simple sugars, both of which can be produced via lipase, are useful because of their low toxicity and high biodegradability [112]. Biodiesel manufacturing employing oleic acid, microalgae, and vegetable oil is a well-established example of the widespread use of lipase-mediated catalysis [113,114]. Using lipase-mediated esterification eliminates the need for harsh solvents and speeds up the reaction time for making biodiesel, two common issues with traditional techniques.

3.3. ***Aminolysis:***

Aminolysis, shown in Figure 3, breaks down a substance by interacting with ammonia or an amine molecule, which adds or replaces an amino group. Methods involving biological and chemical processes are typically used. Since it allows for the synthesis of a wide variety of chirally pure compounds, lipase-mediated aminolysis has significant uses in the pharmaceutical industry [115]. Sphingomonas sp. SpL, a recently found intracellular lipase with outstanding properties, maybe a useful biocatalyst for the efficient and environmentally friendly production of amides. To speed up the kinetic resolution of many aromatic alpha-hydroxy acids, a novel enzymatic aminolysis strategy has been developed. This technique, which uses anhydrous ammonia as the chiral derivatizing agent, is suitable for use in non-aqueous fluids at room temperature. Additionally, it is used in the manufacture of antimalarials [116], fibromyalgia syndrome [117], milnacipran [118], and hormone receptors [119]; beta-amino acid esters are the fundamental components of many pharmaceutical medications [120][121]

3.4. Transesterification:

Figure 3 shows how transesterification sequentially exchanges the R group of an ester molecule for the R group of an acid, alcohol, or another ester. Food waste oils are cheap, non-edible, and abundantly available, making them ideal for biodiesel fuel synthesis [122-124]. Transesterification of diols with dimethyl carbonate (DMC) using immobilized lipase B from Candida antarctica can be used to greenly synthesize cyclic carbonates, the building blocks of polyurethanes and polycarbonates. Polyurethanes harden or soften foams. DVDs and impact disks use polycarbonates [125]. It's intriguing that lipase-mediated trans-esterification can reduce wasted cooking oil's high cytotoxic capability in bioremediation [126].

4. Microbial production of lipases:

Microbial lipases have attracted more interest from the industrial sector than other types of lipases because of their high selectivity, stability, and versatility in terms of substrates [127]. Lipases can be produced by a wide variety of microorganisms, including bacteria, fungi, and yeast.

4.1 Bacterial lipases:

The lipase enzyme is produced by a wide variety of bacteria, both Gram-positive and Gram-negative. Bacillus subtilis, Bacillus licheniformis, etc are some of the most commercially important lipase-producing bacteria, however pseudomonas, Burkholderia, and Staphylococcus are also listed as better lipases [128]. Oil refinery byproducts, vegetable oil processing facilities, dairy farms, pulp, and paper mills, and oil-contaminated soil are just some of the environments that have been identified to harbor lipase-producing bacterial strains[129]. Pulp and paper mill sectors were the sources of eight distinct

lipase-producing bacteria, which were discovered by Tripathi et al. (2014) [130]. Five distinct lipase-producing bacterial strains were recently discovered in petrol-contaminated soil by Bharathi et al. (2018) [131]. Previous studies have shown that oil-contaminated environments are ideal for isolating lipase-producing bacterial strains. Lipases are preferred to other chemical or synthetic catalysts because they are safe for humans and the environment. As a result, they are popular in the fields of food, dairy, flavouring, detergent, medicine, biofuels, and cosmetics [131][132].

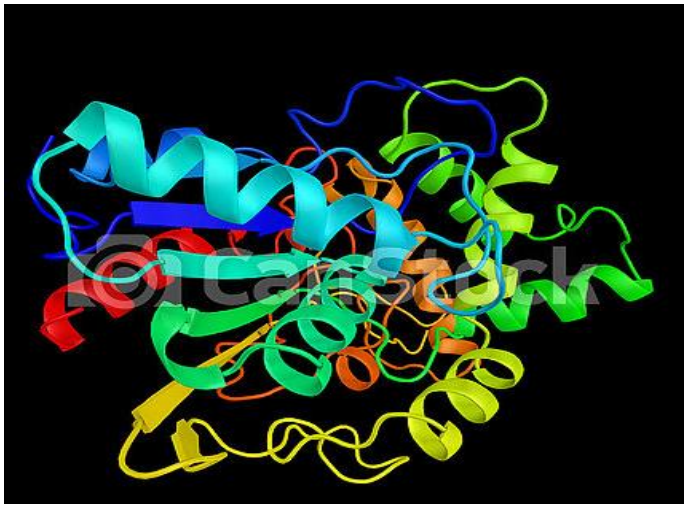


Figure 4: Image of bacterial lipase (Hydrolase family)

4.2 Fungal lipases:

Potential lipase producers from fungal strains are well known, as they possess crucial commercially-relevant characteristics such as unusual catalytic capabilities [133]. Fungi from the genera Rhizopus, Aspergillus, Penicillium, Geotrichum, and Mucor produce the vast majority of the world's economically and industrially significant lipases. Lipase synthesis by fungi is sensitive to factors like strain, carbon and nitrogen sources, temperature, and pH of the growth medium. Filamentous fungi are among the best microbiological sources for lipase production because they are easy to cultivate and harvest, and even easier to extract, purify, and process. The Aspergillus aculeatus strain was identified by Roy et al. [134] and shown to produce an exceptionally high amount of lipase (9.51 U/ml) from soil contaminated with dairy manure. New fungus strains are being isolated and selected due to the increasing demand from the industry for lipases with varying catalytic characteristics.

Figure 5: Image of Fungal lipase from Thermomyces lanuginosa

4.3 Yeast lipases:

Yeast-derived lipase finds specialized use in the chemical, pharmaceutical, and biodiesel industries. Recent research suggests that the best and principal lipase producers include the yeasts Candida 6

utilis, Candida rugosa, etc. Literature suggests that among yeasts, Candida sp. has the most potential as a lipase producer. [135]

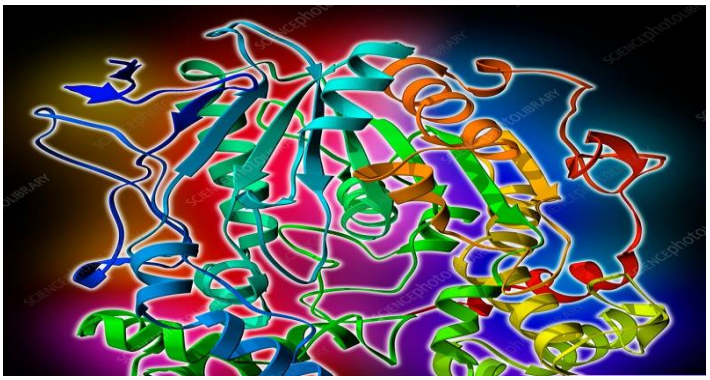


Figure 6: Image of yeast fungus lipase

Table 1: The occurrence of lipases in different microorganisms [135]

Organism	Author name	Reference number
Pseudomonas cepacian	Sugihara et.al	136
Pseudomonas aeruginosa	Kukeraja et.al	137
Staphylococcus sp	Ajith kumar et.al	138
Achromobacter lipolyticum	Khan et.al	139
Bacillus cereus	Ananth et.al	140
Bacillus licheniformis VSG1	Sangeetha et.al	141
Bacillus smithii BTMS 11	Ephraim et.al	142
Bacillus megaterium AKG-1	Sekhon et.al	143
Bacillus stearothermophilus MTCC 37	Saba et.al	144
Bacillus pumilus RK-31	Kumar et.al	145
Candida Antarctica ZJB09193	Liu et.al	146
Candida rugosa	Pereira et.al	147
Lactobacillus brevis	Chander et.al	148
Lactobacillus lactis	Uppada et.al	149
Lactobacillus plantarum	Lopes et.al	150
Enterococcus faecium MTCC 5695	Rama Krishnan et.al	151
Kluyveromyces marxianus	Deive et.al	152
Lactobacillus delbrueckii subsp. Bulgaricus	El-Sawah et.al	153
Lactococcus heleveticus	Rashmi rt.al	154
Aspergillus niger	Faloni et.al	155
Aspergillus awamori	Basheer et.al	156
Aspergillus fumigatus MTCC9657	Rajan et.al	157
Fusarium solani FS-1	Maia et.al	158
Bacidiobolus	Okafor et.al	159
Thermomyces lanuginosus	Li et.al	160

5. Structure of bacterial lipases:

Other enzymes, including as esterases, proteases, dehalogenases, etc., share structural similarities with lipases and are therefore classified as members of the alpha-beta hydrolase family. Most bacterial lipases found in the extracellular milieu are glycoproteins, while a subset is lipoproteins (161). One serine residue (the nucleophile), one aspartic or glutamic acid (the catalytic acid), and one histidine residue (the histidine) make up the highly conserved catalytic triad in the active site of lipases. In the catalytic triad of lipases, glutamic acid plays a unique role; it is absent from all other alpha- and beta-hydrolytic enzymes. A study of the amino acid sequences, 3D structures, and pH-dependent electrostatic signatures of lipases reveals that the majority of the residues in the active site are non-polar (162,163). In contrast, lipases have a lid domain that can move around to cover the active site when it is not in use. The lid partially opens in the presence of a hydrophobic layer but remains closed in pure water conditions, hence regulating enzyme activity. Compared to mesophilic lipases, which

contain lid domains that are either a loop or a helix, thermostable lipases have larger lid domains with two or more helices (164)

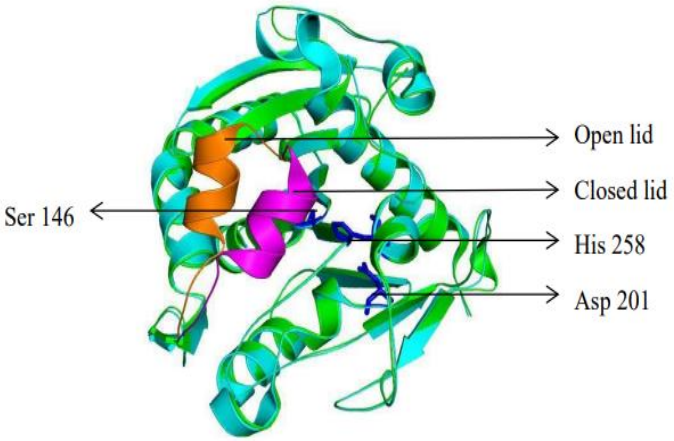


Figure 7: Structure of *Thermomyces lanuginosus* lipase- with catalytic triads [164]

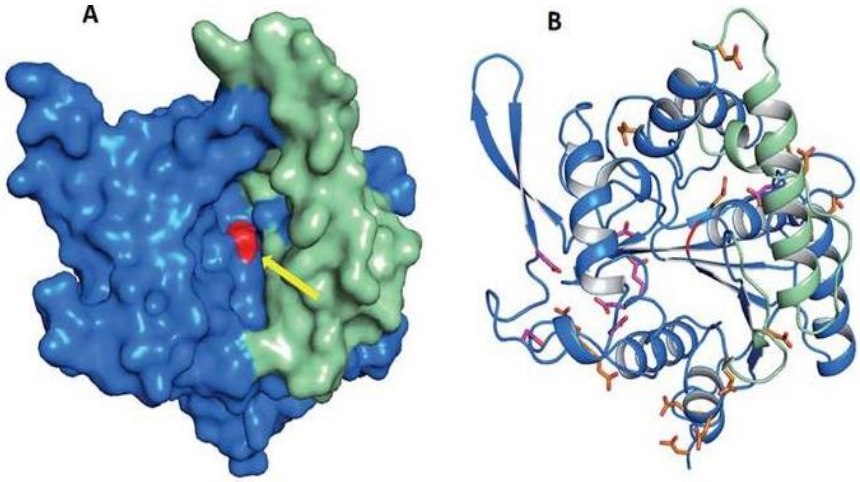


Figure 8: 3-D structure of Lipase

6. Biodiversity of lipases:

Lipases are produced by a wide variety of organisms, including animals, plants, and bacteria. The vast majority of microbial lipases are secreted from the bodies of bacteria (165) and fungi. Bacteria produced the highest number of lipases (166), whereas algae produced the lowest. (Table 2).

Table 2: percentage (%) of lipases produced from different organisms [166]

S.No	Source	Percentage (%) of lipases
1.	Bacteria	45.0
2.	Fungi	21.0
3.	Animals	18.0
4.	Plants	11.0
5.	Algae	03.0

7. Properties and characteristics of lipases:

Lipases are thought to be single-chain proteins with a molecular weight between 19 and 60 kDa. The physical behavior of lipases is affected by a number of factors, including the fatty acid's position on the glycerol molecule, the length of the fatty acid chain, and the degree of unsaturation [167,168]. These factors also affect how a certain triglyceride performs and how it makes you feel from a nutritional standpoint. Some lipases are soluble in organic solvents, making them useful catalysts for

a wide range of processes. [169] One example is esterification. Lipases have activities that change depending on the pH level; they are stable between 7.0 and 4.0 and 8.0. In contrast to the alkaline lipases produced by P. nitroaeducens, which are active at a pH of 11.0 [170], the extracellular lipases produced by Chromobacterium viscosum, A. niger, and Rhizophus sp. are active at acidic pH. Even in the absence of water, lipases have been shown to reverse esterification and interesterification processes [171,172] under laboratory conditions. Although cofactors are not required for the production of lipase activities, calcium is the divalent cation that enhances lipase activity [173,174]. Zn2+, Mg2+, EDTA, and sodium dodecyl all had a modest inhibitory effect on lipase activity. Temperature stability profiles show that lipase half-lives are more stable at low temperatures [175,176]. Using the aryl glycerol substrate, it was possible to classify lipases into two categories, one of which is region-insensitive and the other of which is region-selective. The second class of lipases selectively hydrolyzes acylglycerols at positions 1, 3, and possibly elsewhere to liberate fatty acids [177-179]. These enzymes have properties that make it possible to isolate esters and alcohols in their optically pure forms. When water activity is low, using an organic medium is superior to switching to a different solvent [180]. Therefore, modifying the properties of the solvent can change the enzyme's specificity. Enzyme catalytic efficiency is highly sensitive to the solvent it is in contact with because of the enzyme's delicate structure [181,182].

8. Therapeutic applications of lipase:

Lipases have been used in both the pharmaceutical and healthcare sectors. Lipase-catalyzed enantioselective inter-esterification and transesterification reactions for selective acylation and de-acylation operations are significant in the pharmaceutical industry. Monoglycerides are commonly used in pharmaceutical emulsifiers, and these lipids can be altered with lipases. Pancreatic lipases, proteases, and amylases have been used successfully to treat fat malabsorption in HIV patients. Lipases, amylases, and proteases were used together to treat patients with pancreatic enzyme deficits. Determining the presence of acute pancreatitis or pancreatic damage using serum lipase levels is a useful diagnostic tool [183].

Malignant tumors, dyspepsia, gastrointestinal issues, and the cutaneous manifestations of digestive allergies have all been treated with lipases because of their ability to activate TNF. In order to develop lovastatin, a drug that lowers blood cholesterol levels, scientists turned to the lipase enzyme discovered in Candida rugosa. [184].

8.1 Lipase Inhibitors for Obesity:

Obesity has been a problem for centuries. Obesity is more than simply an aesthetic problem because of the abnormal physiology that develops as a result of being overweight. Obesity is a risk factor for a wide range of serious medical disorders, including cardiovascular disease [185], hypertension [186], elevated cholesterol [187], and type 2 diabetes [188] (and their related complications]. Type 2 diabetes, gallstones, and metabolic syndrome are more common in the obese than in the normal population [189,190]. Besides its deleterious effects on reproduction, obesity is associated with an increased risk of cardiovascular disease, hypertension, osteoarthritis, and gout [191,192].

There is a strong correlation between obesity and gynecologic malignancies such uterine cancer, breast cancer, colon cancer, and others. As a result, reducing the prevalence of chronic metabolic illnesses and the deaths they cause requires a concerted effort to prevent and cure obesity. Obesity can be treated in a number of ways, from making moderate lifestyle modifications to more extreme ones like medication or surgery (liposuction). Unwanted fat clumps after liposuction are a reality that patients have to face. Liposuction has been shown to have no effect on subcutaneous fat, or at most a very small effect, and still presents certain risks for patients, according to research. Most current methods of weight loss use a combination of lifestyle modifications (such as food and exercise) and shorter pharmaceutical regimens (to reduce side effects and maximize benefits). Pancreatic lipase (PL) inhibitors are essential for human fat metabolism. It functions as a metabolic enzyme, breaking

down the oil consumed into usable glycerol and fatty acids [193]. When PL is inhibited, some of its breakdown capacity is lost, and the source of fat entering the circulation is controlled, resulting in a reduction of lipids.

8.2 Factors contributing to obesity: high-fat diets and the enzyme lipase:

Modern Western society's obesity epidemic may be traced back to two main causes: overeating and inactivity. Excessive amounts of fat in the body are the fundamental reason for obesity [194,195]. Excess lipid accumulation is associated with the development of non-alcoholic fatty liver disease and white adipose tissue hypertrophy [196]. The accumulation of risk factors related to obesity and hyperlipidemia is connected with increased mortality rates. These risk factors include insulin resistance, worsening liver fibrosis, decreased glucose tolerance, and hypertension. Evidence from studies of persons with type 2 diabetes demonstrates that insulin resistance in the muscles and the accumulation of intramyocellular lipids precede insulin resistance in the liver and the development of the disease [197]. Problems in fat metabolism have been connected to the development of obesity. This combination of dietary fats accounts for 90 percent of an individual's caloric intake per day. Absorption of exogenous fat requires hydrolysis because it is not useful in its natural state. Digestive lipases are abundant and can be found in a variety of organs and tissues, including the tongue, stomach, and pancreas. As a critical regulator of pancreatic lipase release, gastric lipase is widely thought to play a crucial role in lipolysis [198,199]. The lipase produced in the pancreas is the most important since it regulates how well fats are absorbed by the body. The pancreas secretes the digesting lipase known as pancreatic lipase (PL). This enzyme converts oil triacylglycerol substrates into monoglycerides and free fatty acids. The colon's enterocytes transport monoglycerides and free fatty acids to the intestines for digestion [200,201]. Fatty meals increase 1,2-glycolide and fatty acids because lipase breaks triglyceride-based lipids into monoglyceride, glyceryl ester, and free fatty acid. Lipoproteins and cholesterol cannot be synthesized without gastric (10-30%) and pancreatic (50-70%) lipases hydrolysing them into free fatty acids and monoacylglycerol in the stomach and small intestine. As a means of storing energy, adipose tissue takes in lipid-mixed particles like bile acid and re-synthesizes triacylglycerol.

8.3 The role of lipases in the development of coronary atherosclerosis:

The risk of developing atherosclerosis is regulated in part by lipoprotein lipases, endothelium lipases, and hepatic lipases. The presence of cholesterol in the blood and particles that carry cholesterol in the bloodstream contribute to the development of atherosclerosis. LDL molecules are responsible for transporting the insoluble cholesterol produced in the liver to the active cells that can absorb lipids. More HDL particles in the blood mean more cholesterol can be broken down and eliminated by the liver, lowering the risk of cardiovascular disease. People with optimal HDL and LDL levels are protected from heart disease.[202]

8.4 Possible role for lipases in anti-cancer therapy:

Sedentary behaviour and high-calorie diets have been related to several types of cancer, including breast cancer, pancreatic cancer, liver cancer, colon cancer, and prostate cancer [203]. Lipoprotein lipases, enzymes that speed up the hydrolysis of plasma TG, are now known to have a role in the development of pancreatic and colon cancer and precancerous lesions. Hepatocellular carcinoma has been connected to the deletion of a gene on the short arm of chromosome 8, which is known to be a tumor suppressor gene in humans. The FISH investigation further verifies that a lack of LPL is associated with an increased risk of prostate cancer.

The human breast cancer 2, liver cancer 1, and mitochondrial tumor suppressor 1 (MTS1) genes are all found on chromosome 2's short arm (MTUS1). Thus, the LPL gene loss on this chromosome affects the proximal cancer-related genes at issue, and the sum of these effects is said to produce carcinogenesis [203].

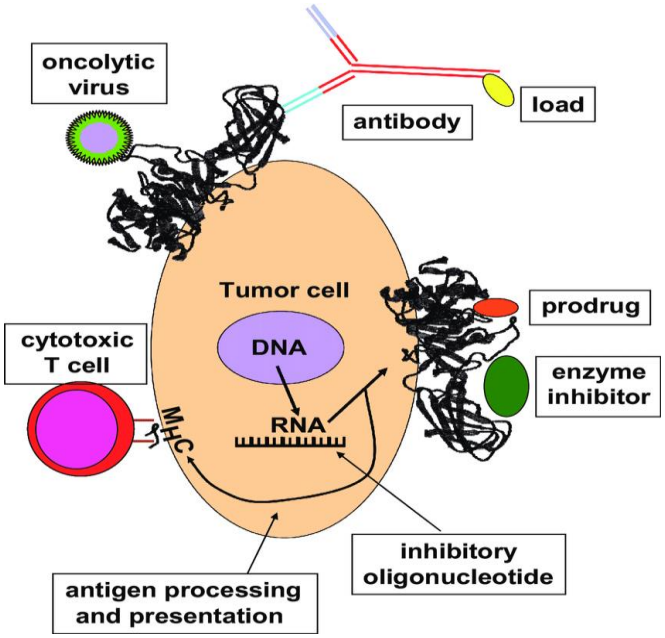


Figure 9: Lipases as potential cancer treatments [204]

The regulation of TGs and monoglycerides by LPL is crucial for lipid and lipoprotein metabolism. Cachexia-inducing LPL modulators, such as tumor necrosis factor and interleukins (IL-1 and IL-6), reduce LPL function, resulting in decreased fat accumulation in organs [205,206]. These results pave the way for future studies aimed at enhancing LPL activity to reduce cachexia in cancer patients. Although dietary therapies failed to cure the wasting caused by LPL suppression in the cachexia paradigm, the LPL activator NO-1886 had a positive effect in both the LPL suppression model and the Leydig cell malignancy model in rats. [207-210]

8.5 Lipoprotein Lipase and Its Clinical Implications:

A widely established method of preventing hyperchylomicronaemia-induced pancreatitis, the development of therapy techniques to reduce significantly elevated triglyceride levels has been the quickest path to the market for triglyceride-lowering medications. However, evidence that lowering triglyceride levels reduces pancreatitis incidence was absent until now [211]. Volanesorsen, an antisense oligonucleotide inhibitor of ApoC3 synthesis, has its trial results published by Witztum et al. [212]. The levels of circulating ApoC3 and average triglycerides were both decreased by 84 percent and 77%, respectively, after treatment with volanesorsen in double-blind research including 66 individuals with hereditary hyperchylomicronaemia syndrome. The authors point out that while the treatment group only experienced one incidence of pancreatitis, the control group experienced four. In addition to lowering platelet counts, volanesorsen was linked to an increase in the frequency of cases of local injection site responses. Some newer antisense formulations are being developed with the intention of solving these problems.

Kanter et al. [213] observed that ApoC3, independent of circulating triglycerides, was a risk factor for CVD in type 1 diabetics, supporting the recommendation to decrease ApoC3. In a mouse model, ApoC3 antisense treatment reduced atherosclerosis, demonstrating that this apoprotein has a detrimental effect beyond its effects on LpL and hepatic lipoprotein absorption. Thus, decreasing ApoC3 may offer protection beyond triglyceride reduction. LDL or residual lipoprotein ApoC3 levels may also indicate clinical CVD risk. This effect of ApoC3 on the hepatic clearance of TRL via syndecan 1 was analyzed by Ramms et al. [214]. (SDC1). Both apoE-mediated TRL clearance via LDLR/LRP1 and SDC1-mediated TRL clearance are lost in apoE/SDC1 null mice. They discovered that higher LpL activity in white adipose tissue, brought about by reducing ApoC3 in the absence of apoE expression, resulted in a striking reduction of plasma triglycerides.

Ashraf et al. [215] reported dangerously low plasma LpL levels in a 15-year-old girl with intermittent chylomicronemia and GPIHBP1 autoantibodies. Immunosuppressive drugs reduced GPIHBP1

autoantibodies and normalized plasma triglycerides in a patient with chylomicronemia and severe pancreatitis. The authors suggest testing for GPIHBP1 autoantibodies in acquired chylomicronemia patients with unknown causes.

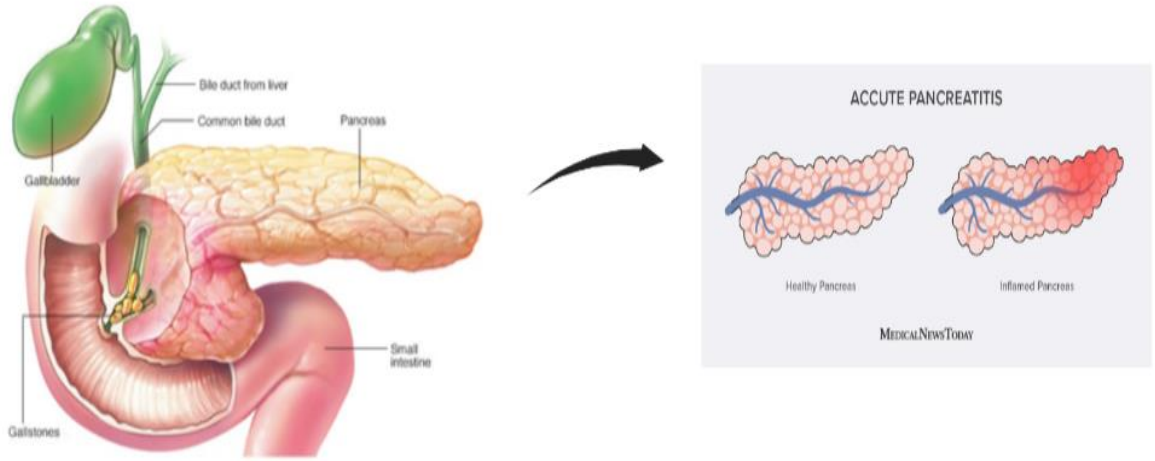


Figure 10: Image of Pancreatitis: healthy and inflamed pancreas

8.6 Newer Approaches to reduce hypertriglyceridemia:

Hyperchylomicronaemia syndrome is characterized by recurring pancreatitis or the need for a patient to adhere to an extremely restrictive diet for the remainder of their lives, but new therapeutic options are on the horizon. For patients without LpL genetic abnormalities, two additional treatments are in the works, in addition to lowering ApoC3. A new ApoC2-like peptide decreases blood triglycerides, as demonstrated by Wolska et al. [216]. Removing ApoC3 from circulating lipoproteins has this impact, and it's no surprise that lowering triglyceride levels in an LpL-deficient model also works, as both of these compounds do the same thing.

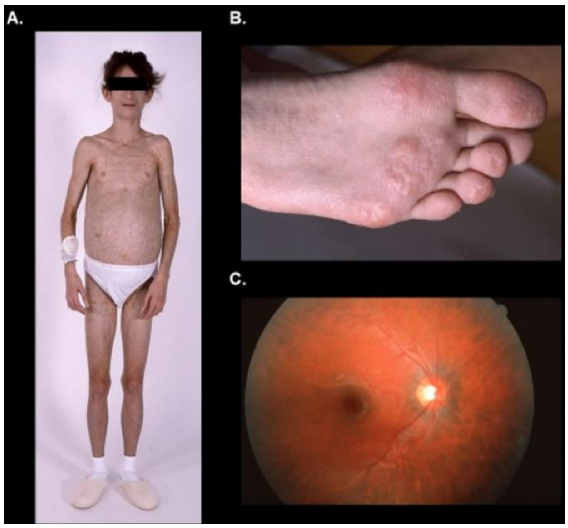


Figure 11: Image of hypertriglyceridemia

8.7 Pharma: stereo- and regio-selective resolution:

In the kinetic resolution of chemicals, lipases are often utilized enzymes. Racemic alcohols and kinetic racemic combinations of substances like flurbiprofen (C. antartica lipase Novozyme® 435) and N-hydroxymethyl vince lactam can be separated using the regioselectivity of lipases. Herbicides (phenoxypropionate) can be made through the resolution of 2-halopropionic acids and the esterification of (S)-isomers in butanol and hexane, both of which are interesting applications of lipase

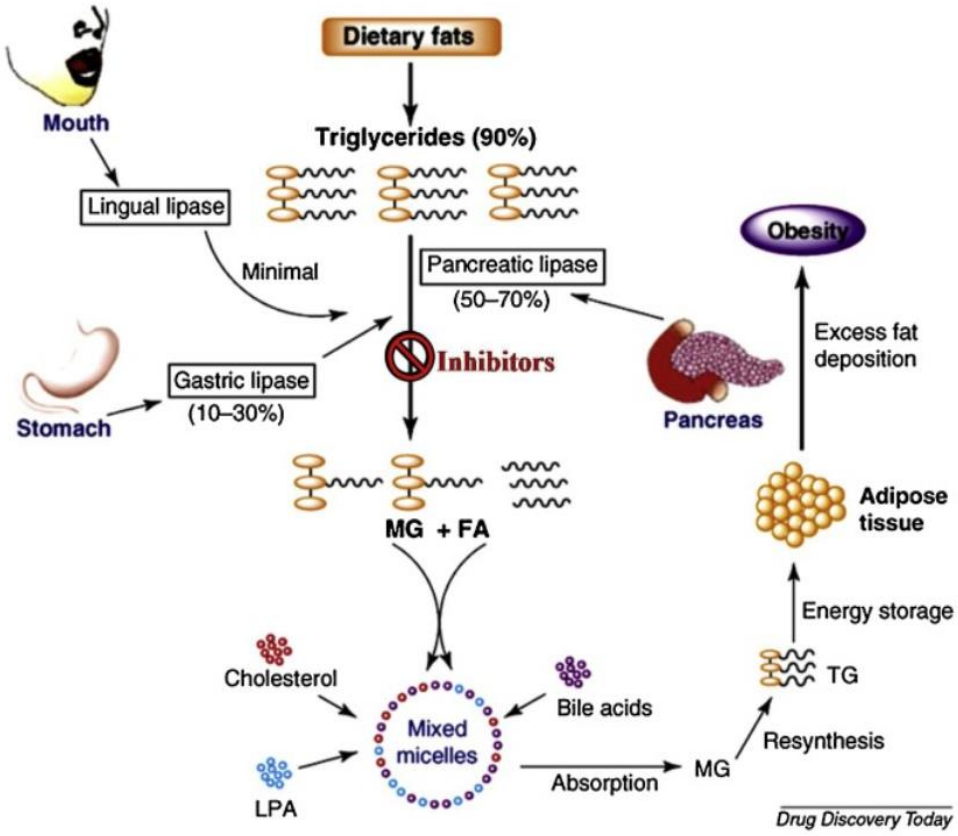
resolution.

The regioselectivity of lipases has been exploited for the production of molecules that are challenging to manufacture chemically. Sugar esters, which are both non-ionic and biodegradable, find widespread use in many different types of cleaning agents, medicines, and dental care products. Because sugars are poorly soluble in organic solvents and require high temperatures and pressures to be esterified with fatty acids, standard methods of producing them are challenging. Candida antarctica lipase B (immobilized as Novozyme® 435) in DMSO and acetone (1:10 v/v) were used to accomplish this esterification. With this two-solvent setup, we were able to get a 64% yield from the xylose caproate ester. [217]

9. Lipase as probiotics:

9.1 Disrupting pancreatic lipase (PL) metabolism by several means:

A diagram of the human body's lipid metabolic system is shown in Figure 12 [218]. Lipase inhibitors change the structure of stomach/trypsin in the presence of active lipase, resulting in a decrease in lipids like triglycerides. Hydrolysis can be helpful in the management and treatment of obesity because it reduces energy expenditure during digestion and absorption of dietary lipids and also prevents the buildup of adipose tissue [219]. In most cases, the lipase inhibitor and the bound lipase are removed from the system once they have served their purpose. Consequently, it does not leave any sort of physical mark on people.



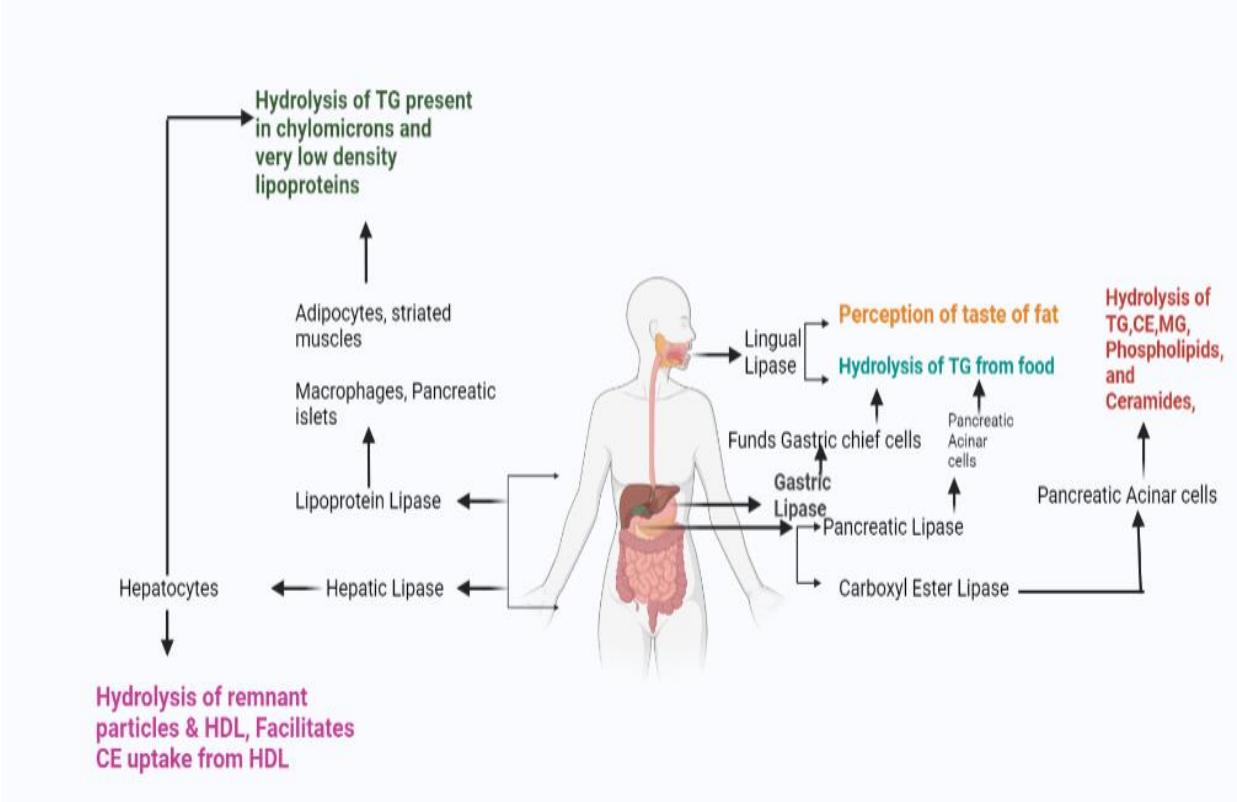
Pancreatic lipase's metabolic pathways are depicted in Figure 12 [194]

Commonly recommended weight loss drugs can be split into two groups: central appetite suppressants and peripheral lipase inhibitors. Orlistat and similar medications block the absorption of fat in the intestines, which can help people lose weight. Headaches, dizziness, dry mouth, bitterness, constipation, and sleepiness are merely some of the side effects that have been associated with the use of these drugs in a number of clinical trials. This appetite suppressant, which is mostly made up of fenfluramine and sibutramine, blocks the transmission of certain impulses across the central nervous system. Further, these medications may cause a wide range of mental or cardiovascular

adverse reactions, reducing their clinical utility or possibly leading to their withdrawal from the market. Peripherally acting lipase inhibitors are safer because they don't enter blood vessels or the nervous system and don't affect mineral balance or bone circulation. Consequently, using lipase inhibitors is nothing to be overly concerned about.
Clinical trials have indicated that the pancreatic lipase inhibitor orlistat is effective at reducing high-fat diet-induced obesity. Nonetheless, orlistat use has been associated with oily bowel movements [220-222].

9.2 Lipases and their role in fat digestion:

In order to break down dietary lipids, particularly triacylglycerol, several different cofactors, bile salts, and enzymes must be transported through the digestive tract. Triacylglycerol molecules have a 3′-unsaturated fatty acid chain attached through an ester bond to the glycerol backbone. Mouths release digestive enzymes, including lipases, to break down food; later, in the stomach, acidophilic proteins take over. Human stomach lipases, lingual lipases, etc. are two of several lipase varieties found in the digestive system.



Clinical applications and anatomical functions of lipase (Figure 13)

9.3 Digestive lipase:

Lipases are digestive enzymes generated by the body that cleave lipids into smaller pieces. The lack of digestive lipase can be seen in the early stages of gastrointestinal distress, dyspepsia, and the cutaneous symptoms of digestive allergies, as well as in diseases like cystic fibrosis and chronic pancreatitis. Lipases are helpful digestive aids that might reduce some symptoms.

Pharmaceutical uses for enzymes like hyaluronidase and thiomucase to treat skin irritation have already been patented. Because of their ability to detect infectious and illness conditions, lipases are now being used as diagnostic tools in the pharmaceutical business. Using specific lipases and a colorimetric enzyme assay to identify the glycerol produced during digestion, serum triglycerides can be quantified enzymatically. Serum lipase concentrations in the blood are useful for diagnosing and identifying cases of acute pancreatitis and pancreatic injury.[223]

10. Methods for Identifying Lipase Producers:

Agar medium and liquid media with diverse substrates have been used to identify lipase-producing bacteria. The capacity to produce lipase can be screened for in two separate ways: directly and indirectly.

10.1. Direct method (qualitative- on agar medium):

To identify lipolytic bacteria, solid agar plates supplemented with substrates or indicator colors can be utilized. When testing different microbes for their lipase enzyme synthesis, this quick screening procedure is quite helpful. Direct plate assay is further subdivided into two categories based on the usage of substrates and indicator dyes. [224]

10.1.1. Evaluation of different lipid substrates using gel diffusion tests:

These techniques are utilized for the screening of lipolytic microbial strains based on their capacity to degrade the lipids embedded within the solid medium, resulting in the formation of a distinct lipolysis zone. Different agar plate tests have been developed depending on the substrates that are used. [224]

10.1.2. Indicator dye gel diffusion assays:

Indicator colors and substrates of varying types are used in these techniques to identify lipase-producing bacteria. Indicator dyes are used for screening, and they include phenol red, Rhodamine B, victoria blue, and night blue. Hydrolysis of substrates followed by reactions with fluorescent dye produce glowing halos around microbial colonies, that are visible under UV light. [224]

10.2. Indirect method:

The ability of a microorganism to produce lipase can be quantified using a number of different quantitative approaches. These include the titrimetric approach, the spectrophotometric approach, the chromatographic approach, and molecular screening approaches. More than fifty years ago, lipase activity was often estimated using titrimetric methods.

11. Modulators of Lipase Activity:

There are many factors that influence the rate at which lipase enzyme is generated. Most importantly, when an inducer is present, carbon supplies, nitrogen sources, and temperature all come into play.

11.1. Carbon source:

Lipase in microorganisms is typically created by inducing lipase-generating genes. Olive oil, among other carbon sources, contributes significantly to the activation of all microbial lipases. The induction of lipases, however, with oil-carbon sources have influenced the recovery. Aspergillus terreus, when fed mustard seed oil as a carbon source, produces lipase at a high rate [224]. Lipase enzyme synthesis in fungi is boosted when olive oil cake and sugar cane bagasse are used together. Researchers have shown that olive oil stimulates lipase synthesis in bacteria more than other oils.

11.2. Nitrogen source:

Having access to a reliable nitrogen source is crucial for the development and improvement of microorganisms. Producing higher lipases from all kinds of microbes requires a variety of organic and inorganic nitrogen sources, including ammonium salts, yeast extract etc. When urea was included in the medium used to cultivate Rhizopus sp., the resulting lipase had increased lipolytic activity. Furthermore, Aspergillus sp. has been shown to produce lipase when peptone is combined with another nitrogen extract [224].

11.3. Temp influences lipase activity:

Lipase synthesis in microorganisms is similarly sensitive to temperature. The secretion of enzymes in the shaking flask method is critically dependent on the optimum temperature. At 37˚C, there was

a greater concentration of lipase in the biomass. Upping the temperature by just a few degrees, to around 38 degrees Celsius, has been shown to increase lipase synthesis, according to the research. Lipase enzyme synthesis is suppressed at lower temperatures, and lipase enzyme activity can be affected by temperature changes. [224]

11.4. The pH-Dependent Impact on Lipase Activity:

Most bacterial lipases have either alkaline or neutral pH optima. Scientists found that lipase synthesis in bacteria and yeast was increased in alkaline and neutral pH environments. The formation of fungal lipases, on the other hand, is stimulated by a more acidic pH. [224]

12. Lipase as Immunomodulators:

Immunological and neurological processes are regulated by lysophosphatidylserines (lyso-PSs). In vivo, lyso-PS metabolism is unclear. SS Kamat et al.[225] found that ABHD12 is a significant brain lyso-PS lipase, implicating lysoPSs in the neurological disorders polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC), which are caused by ABHD12 gene null mutations. They use activity-based profiling, pharmacological, and genetic approaches to identify the poorly described enzyme ABHD16A as mammalian phosphatidylserine (PS) lipase that creates lyso-PS. A small-molecule ABHD16A inhibitor depletes lyso-PSs from cells, including PHARC lymphoblasts. Disrupting ABHD12 and ABHD16A in murine macrophages increases and lowers lyso-PSs and lipopolysaccharide-induced cytokine production. Abhd16a(-/-) mice had lower brain lyso-PSs than Abhd12(-/-) mice. Thus, an ABHD16A-ABHD12 axis dynamically regulates lyso-PS metabolism in vivo, making these enzymes prospective targets for neuroimmunological diseases.

For nutrition acquisition, colonization, and infection, commensal and pathogenic bacteria hydrolyze host lipid substrates through secreted lipases and phospholipases. Bacterial lipase action on mammalian lipids and phospholipids can release free fatty acids, detoxify antimicrobial lipids, and dissolve membranes. Sal1 and Geh, secreted by the gram-positive bacterium Staphylococcus aureus, hydrolyze ambient lipids with specificity for short- and long-chain fatty acids, respectively. X Chen et al.[226] discovered that S. aureus Geh suppresses innate immune cell activation. They studied whether S. aureus lipases interact with the host immune system to impair innate immune recognition. Geh lipase, but not other S. aureus lipases, suppresses innate cell activation in culture. Mutating geh increases proinflammatory cytokine production, innate immune activity, and bacterial clearance in infected tissue. These in vitro and in vivo impacts on innate immunity were caused by ester hydrolysis of S. aureus lipoproteins, a prominent PAMP of extracellular gram-positive bacteria. These experiments show how targeted silencing of a broadly conserved PAMP hides pathogen detection by innate immune cells.

13. Conclusion:

Because of their versatility as biocatalysts and their prevalence in the body, lipases find widespread usage in industry. They serve a vital physiological function. Due to the obstructive effects of edible oil and the dairy industry on biochemical processes, lipids and other compounds suitable as substrates for lipolytic enzymes are released into the environment during food processing. This reduces the activity of the biomass and causes problems for the biochemical processes. In comparison to traditional lipid degradation techniques, microorganisms have the potential to biodegrade lipid waste in mild circumstances effectively, creating lipolytic enzymes and promoting environmental sustainability. In the contemporary environment, the hydrolysis of synthetic plastics with ester bonds, pesticides, insecticides, and parabens is one new development that is also used to produce bioenergy and save energy in order to support the world's hazardous wastes. The creation of high-value-added goods employing enzymatic catalysis linked to microbial lipases, which use less energy, is another crucial factor. Lipases have expanded and are currently evolving quickly as leading options for the design of therapeutic and diagnostic aids. Lipase enzymes are employed primarily for treating lifestyle illnesses like obesity in pharmaceutical and therapeutic applications as modulators, such as activators and inhibitors. The study of lipases has grown and is quickly becoming a contender for the

creation of therapeutic and diagnostic tools. In pharmaceutical and medical applications, lipase enzymes are used as immunomodulators, including activators and inhibitors, with a focus on treating lifestyle diseases like obesity. Thus, the usage of these lipases has a significant positive impact on a wide range of biotechnology-based goods. Modulators currently have a significant impact on medicines and will likely continue to grow in the near future. Hence, utilizing these lipases significantly improves a wide range of biotechnology-based productions.

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