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Enhancement of *Geobacillus thermodenitrificans* Lipase Activity and Binding Affinity by Acetone Treatment: Kinetic and *in silico* Investigations

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Abstract: Lipase *Geobacillus thermodenitrificans* (LipGt) is a thermostable enzyme that has an optimal activity temperature of 65°C. It can tolerate various organic solvents and has an improved activity, especially in the presence of acetone. In this study, in-vitro and *in-silico* studies were carried out to investigate the effect of acetone in enhancing the kinetic and binding affinity of LipGt. The LipGt was produced in a shake flask and then purified using ultrafiltration, affinity chromatography, and gel-filtration chromatography. Its activity was determined by colorimetric assay through absorbance measurement at 715 nm and comparing it with a standard curve. The Lineweaver Burk plot and molecular docking were utilized to study the kinetic and binding properties between LipGt and olive oil in the presence of acetone, respectively. The findings of our experiment demonstrated that the acetone treatment improved K_m, V_{max}, and K_{cat} of LipGt from 0.18 µM, 2.56 µmoL/mg/min, and 69.1 min^{-1} to 0.30 µM, 4.32 µmoL/mg/min and 116.6 min^{-1}. It indicated that the LipGt had a greater catalytic capacity toward the substrate and a higher reaction rate when it was treated with acetone. In the *in-silico* study, the binding free energy of LipGt was improved by acetone from -4.4 to -5.8 kcal/moL. The number of hydrophobic interactions was doubled with the acetone treatment, which assisted in maintaining the tertiary structure of LipGt and enhancing its activity after 24 h of incubation. Acetone altered the core active site region of LipGt and increased the binding affinity toward olive oil. This study has discovered a basic understanding of the kinetic and binding affinity of LipGt with acetone, attributing it to become a potential biocatalyst in industrial applications that require operation in harsh conditions such as the production of biodiesel.

Keywords: Thermostable Lipase (LipGt), Acetone, Kinetic Parameters, Binding Affinity

Introduction

Lipases are triacylglycerol acylhydrolase (EC 3.1.1.3) that catalyze the hydrolysis of ester bonds to release the fatty acids and glycerols from triglycerides. Microbial lipases are widely utilized in industrial applications such as flavor enhancement, fats and oils modification, wastewater treatment as well as detergent manufacturing. The main reasons for the broad utilization of microbial lipase were due to their cost-effective and easier manipulation during production as well as recovery phases. Besides, they also have a broad range of substrate specificity, regioselectivity, and stereoselectivity (Anuradha *et al*., 2012; Yao *et al*., 2021).

Generally, all the reactions in the industry fields are carried out at a higher temperature to improve the conversion rate and product yield with a minimal contamination risk. The thermostability of the enzyme becomes more significant when considered as an industrial biocatalyst. It is found that the lipases isolated from thermophiles have a greater tolerance towards extreme conditions such as the presence of organic solvents, a wide range of pH, and different salt concentrations, compared to their mesophilic

counterparts. Therefore, the lipases isolated from thermostable microorganisms have attracted more attention from research and industries (Anuradha *et al*., 2012).

Geobacillus was a new genus that previously had been assigned as rRNA group 5 thermophilic *Bacillus* spp. *Geobacillus thermodenitrificans* IBRL-nra is a rod-shaped, Gram-positive thermophile that can grow at the range between 45-70°C with neutral pH conditions. The lipases from *Geobacillus thermodenitrificans* IBRL-nra (LipGt) can work optimally and retain their initial activity for 3 h at 65°C. Based on the previous study, the activity of LipGt can be maintained at pH 7.0 for 16 h. It had approximately 30 kDa of molecular weight which was quite small compared to other lipases. The smaller size of LipGt increased its stability as only small changes occur in its native conformation. LipGt also has a higher solvent tolerance and can be used for various industry purposes. Thus, the effect of various organic solvents on the LipGt activity was investigated, determining its properties for commercial applications in different industries (Anuradha *et al*., 2012-2013).

In the presence of acetone, the activity of LipGt was increased up to 170% after 1 h of incubation and then slightly reduced to 158% after 24 h of incubation. Acetone is a water-soluble solvent that increased the LipGt activity compared to water-immiscible solvents used in the previous study. Anuradha *et al*. (2013). It contradicted the statement that solvents with water remove the essential water from the enzyme, which leads to the unfolding of the molecule and interrupts the active conformation of the enzyme (Lelie *et al*., 2005; Rahman *et al*., 2005). Nevertheless, there was no significant relationship found between lipase stability and the organic solvent types (Ogino *et al*., 2000). From lipase to other, the activity performance was different (Khoramnia *et al*., 2011; Rahman *et al*., 2005; Sugihara *et al*., 1991) and affected by the organic solvents' structure as well as the surface amino acid types of enzymes (Afshin *et al*., 2011; Torres and Castro, 2004).

Further investigation was carried out to determine the contribution of acetone in different aspects as acetone can significantly improve the LipGt activity. The effect of acetone on the kinetic study and binding properties of LipGt has not yet been described. Thus, the objective of the present work was to investigate the improvement of the kinetic and binding affinity of LipGt by acetone. These findings were valuable and resulted in a better understanding of lipase modification using acetone, enhancing the activity for industrial applications in the future.

Materials and Methods

The bacteria used in this experiment was *Geobacillus thermodenitrificans* IBRL-nra from Universiti Sains Malaysia (USM). The sequence of thermostable lipase from *Geobacillus thermodenitrificans* IBRL-nra (LipGt) was obtained from the previous study by Alyaa (2019). The smiles files of acetone and olive oil were retrieved from the PubChem database.

Protein Expression and Purification

The expression and purification of lipase were referred to the method of the previous study reported by Anuradha *et al*. (2012) 3 mL of culture medium that contained 1.0% (w/v) glucose, 1.25% (w/v) yeast extract, 0.45% (w/v) NaCl and 0.10% of olive oil was inoculated by *Geobacillus thermodenitrificans* IBRL-nra. Then, the inoculated medium was incubated for 1 day at pH 6.8 and 65°C with 200 rpm agitation. After incubation, the fermented culture was filtrated and centrifuged at 6000 g for 15 min to obtain the supernatant (crude enzyme). Finally, the crude enzyme was purified using 3 steps: Ultrafiltration, affinity chromatography, and gel-filtration chromatography. The purified LipGt was then collected and employed to study the effect of acetone on lipase activity and kinetics.

Lipase Activity

Lipase activity can be defined as the yield of fatty acid generated through the hydrolysis of oil per minute. The determination of the lipase activity was referred to the modified colorimetry method of Kumar *et al.* (2005). Olive oil emulsion was prepared by mixing the 1% polyvinyl alcohol and olive oil in a ratio of 3 to 1 using a homogenizer. Then, an orbital shaker was utilized to shake the mixture of 2.5 mL of olive oil emulsion, 1.0 mL of culture filtrate, 1.48 mL of 100 mM phosphate buffer (7.0), and 20 µL of 20 mM calcium chloride at 200 rpm agitation for 30 min. 1.0 mL of 6 M hydroxide chloride and 5.0 mL of isooctane were added to cease the catalysis. After mixing, 4.0 mL of upper isooctane layer containing the liberated fatty acid was transferred into a test tube with 200 µL copper reagent (5% (w/v) copper (II) acetate-1-hydrate and pyridine, pH 6.1) and mixed vigorously. The absorbance of the solution was measured at 715 nm to determine the lipase activity, in which the yield of the liberated fatty acid was measured according to the standard curve of free fatty acid which was oleic acid.

Different culture conditions and types of substrates hydrolyzed affected the lipase activity. Based on the previous study of Anuradha *et al*. (2012)), the best substrate that can be hydrolyzed by thermostable LipGt with optimum activity (100%) at 65°C was olive oil. Thus, olive oil was used as a substrate to determine the kinetic and binding properties of LipGt in the present work.

Effect of Organic Solvents on Purified Recombinant Lipase Activity

The effect of various organic solvents on the lipase activity was determined. The organic solvents were added to the enzyme solution with a ratio of 3:1

(Masomian *et al*., 2010). This mixture was shaken and incubated for 2.5 h at 65°C, pH 7.0, and an agitation speed of 200 rpm. The different organic solvents of 25% (v/v) that were tested included: Hydrophobic solvents of benzene, chloroform, isooctane, n-hexane, and n-heptane; while the hydrophilic solvents were acetone, acetonitrile, diethyl ether, dimethyl sulphoxide, ethanol, methanol and propanol. The lipase enzyme activity was tested under assay conditions described in the previous article reported by Anuradha *et al.* (2013). After incubation, the remaining lipase activity was assayed in 50 mM glycine-sodium hydroxide buffer (9.0) at 45°C for 15 min. The lipase activity without the addition of organic solvent determined in the buffer was set as 100% and utilized to compare with other enzyme activity with additives.

Kinetic Study of Thermostable Lipase

After determining the lipase activity against different triacylglycerols, olive oil was shown as the best substrate used under assay conditions. Hence, olive oil was employed as the substrate to measure the enzyme kinetic parameters, K_m, V_{max}, and $K_{cat.}$ In this experiment, the purified lipase (500 µL) was incubated with different olive oil concentrations (S) ranging between 0.1 to 1% (vol/vol). Thereafter, the lipase assay was carried out at pH 7.0, 65ºC, and 200 rpm agitation, and the reaction Velocity (V) was estimated. The Lineweaver-Burk graph (1/V against 1/[S]) was plotted to determine the kinetic parameters (K_m, V_{max}, and K_{cat}) of the enzyme. The experiment was repeated for the lipase assay with acetone. It should be noted that the velocity of the reaction calculated in this experiment was referred to as the initial velocity of the reaction (the measurement of reaction rate was performed immediately after mixing of enzyme and substrate).

Tertiary Structure Prediction

The structure of LipGt was predicted using several online available servers such as SWISS-MODEL (https://swissmodel.expasy.org/) (Waterhouse *et al*., 2018), protein similarity search (https://www.ebi.ac.uk/jdispatcher/sss/fasta) (Madeira *et al*., 2022) and alpha fold colab (https://colab.research.google.com/github/deepmind/alphafold/blob/main/notebooks/AlphaFold.ipynb) (Jumper *et al*., 2021). The predicted structure was then validated by submitting its PDB file to the Structural Analysis and Verification Server (SAVESv6.0) (https://saves.mbi.ucla.edu/). The model quality was evaluated through ERRAT (Chris and Todd, 1993), verify3d (David *et al*., 1997), and check (Laskowski *et al*., 1993). The check analysis is used to analyze the stereochemical properties and accuracy of the predicted model. Additionally, the ERRAT analysis is used to determine the overall quality of the model whereas the verify3d is utilized to evaluate the compatibility between the tertiary structure of the model and its primary sequence (Agnihotry *et al*., 2022). Thereafter, the predicted model with the highest quality was utilized and its secondary structure of the LipGt was analyzed using pdb sum generate (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) (Laskowski *et al*., 2018). The signal p 4.1 server (www.cbs.dtu.dk/services/SignalP/) (Petersen *et al*., 2011) was used to analyze the sequence and find the signal peptide if it presents.

Molecular Docking

Protein-ligand docking between LipGt and olive oil was conducted on AutoDock vina version 1.1.2 (https://vina.scripps.edu/downloads/) (Trott and Olson, 2010) by docking the olive oil into the LipGt to investigate the binding affinity. For acetone treatment, the LipGt was first docked with acetone and then docked with olive oil. The binding affinity of olive oil with LipGT in the presence of acetone was determined. The binding site residues of LipGt were identified using bio via Discovery Studio version 2024 (https://discover.3ds.com/discovery-studio-visualizer-download). The molecular structures of olive oil and acetone were modeled and optimized using Avogadro 2 version 0.7.2 (https://sourceforge.net/projects/avogadro/files/avogadro2/0.7.2/) (Hanwell *et al*., 2012). Next, the PDB files of LipGt, acetone, and olive oil were converted and saved into PDBQT file format for docking preparation. Molecular docking was performed inside a grid box with a dimension of 29×30×27 Å and the center of the box along X, Y, and Z were located at 3.0404, 0.9003, and -2.1619, respectively. For each effective search, the exhaustiveness and number of modes were set to 8 and 1000. The other parameters were set at their default values. The best-docked conformations having the lowest binding free energies were chosen and their interactions were validated using biovia discovery studio version 2024.

Results

Protein Expression and Purification

The LipGt was expressed in express C43 (DE3) pLysS chemically competent cells incubated with Isopropyl-β-d-thiogalactopyranoside (IPTG). The LipGt was purified with a 1.7% yield by 43.3-fold purification. The molecular mass of LipGt was estimated to be 27 kDa based on the SDS-PAGE analysis of the partially purified and purified enzymes (Fig. 1).

Effect of Organic Solvents on Thermostable Lipase

Based on the data obtained, the activity of thermostable LipGt increased by 171% for the first h during the presence of acetone and then decreased

moderately to 153% after 24 h of incubation. Since acetone was the best organic solvent to improve lipase activity, the kinetic and binding affinity of LipGt assay with acetone treatment were studied.

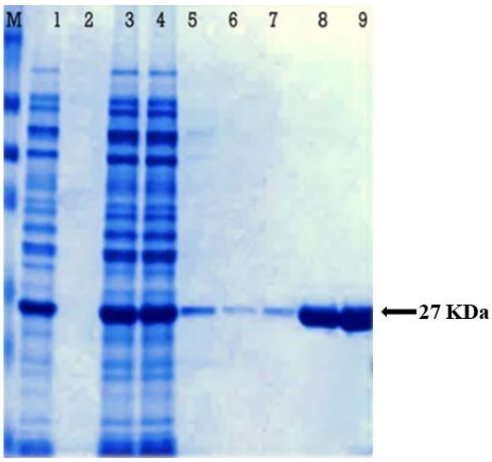
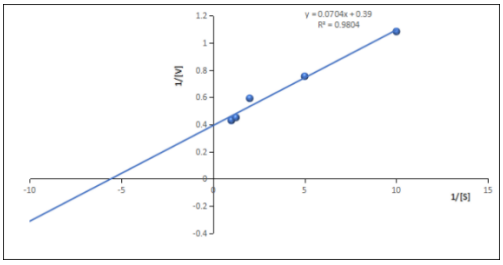
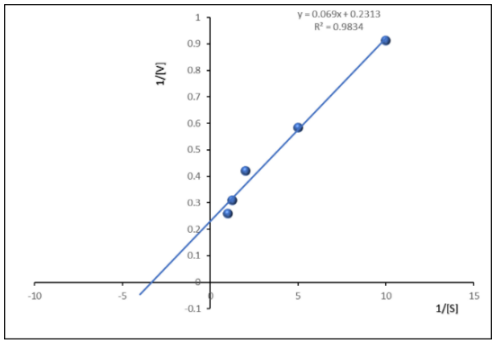


Fig. 1: Purified protein from different purification methods on SDS-PAGE. Lane M: Benchmark protein ladder, lane 1: Induced C43 (DE3) pLysS harboring pET-LipGt, lane 2: Flow through, Lane 3-4: His tag affinity chromatography, Lane 5-7: Second step of gel filtration, lane 8-9: First step of gel filtration. The molecular weight of LipGt was estimated to be 27 kDa due to the presence of the concentrated band



(a)



(b)

Fig. 2: Lineweaver-Burk plots for (a) LipGt assay only and (b) LipGt assay with acetone treatment. Lipase assays were performed using different concentrations of the substrate at pH 7 and a temperature of 65°C. Each value represents the average of 3 independent experiments

Kinetic Study of Thermostable Lipase

For determination of the kinetic parameters, lipase assay was conducted at various substrate concentrations at pH 7.0 and temperature 65°C. The K_m, V_{max}, and K_{cat} for the LipGt assay were determined using olive oil as a substrate. The kinetic parameters for the free enzyme were estimated from the Lineweaver-Burk plot (Fig. 2). The estimated values of K_m, V_{max}, and K_{cat} for the LipGt assay were 0.18 µM, 2.56 µM/mg/min, and 69.1 min^{-1}. Meanwhile, the K_m, V_{max}, and Kcat for the LipGt assay with acetone were 0.30 µM, 4.32 µM/mg/min, and 116.6 min^{-1} (Segel, 1996). All the kinetic parameters of LipGt increased due to the presence of acetone.

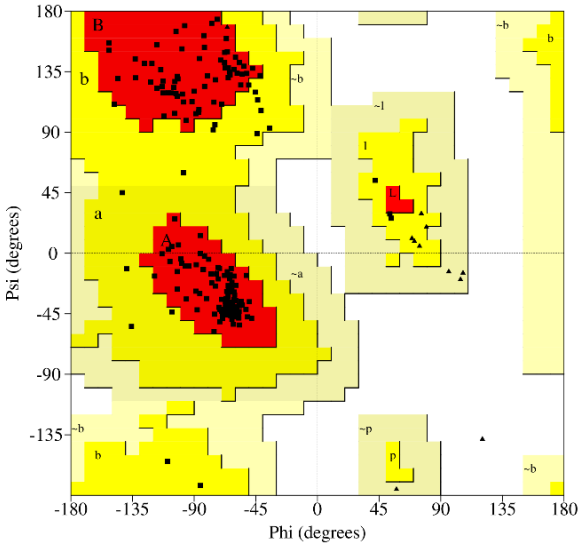
Prediction of Tertiary Structure of LipGt

Among the utilized online servers, the LipGt model predicted by alpha folds Colab had the highest quality based on the Ramachandran plot (procheck).

However, this model failed to verify3d analysis. Besides, the predicted LipGt model contained 265 amino acids and approximately 30 kDa molecular size which was not matched with the result obtained in the SDS-PAGE analysis. When utilizing the signal p 4.1 server, a signal peptide that can regulate the secretion of protein was found at the N-terminal of the LipGt structure. The cleavage site of the putative signal peptide was identified and it was located between valine residue numbers 27 and 28. Thus, the first 27 amino acids which were the signal peptide were removed using PyMOL (https://pymol.org/). After the removal of a signal peptide, the deduced mature LipGt contained 238 amino acids which correspond to the molecular mass of 27 kDa found in the SDS-PAGE analysis. The quality of the deduced mature LipGt structure was improved as it passed all the validation analyses. The Ramachandra plot (Fig. 3) of LipGt showed that there were 92.5% of the residue fell within the most favored region, 7.5% of leaves fell within the additional region and none of the residues fell within the disallowed regions. Additionally, the results of ERRAT and verify3d obtained by the deduced mature LipGt were 98.6% and 81.9%, respectively. After signal peptide elimination, the LipGt shown in Fig. (4) existed as a globule-monomer that adopted an α/β-hydrolase canonical fold, in which a core parallel-β sheet formed by 5 β-strands was surrounded by 12 α-helices.

Molecular Docking Studies

Based on the *in-silico* studies, the binding affinity of acetone with LipGt was -3.4 kcal/mol, and only 1 hydrophobic interaction was found between the acetone and the Tyr 184 of LipGt. The binding affinity of olive oil with LipGt was -4.4 kcal/moL which was decreased to -5.8 kcal/moL in the presence of acetone.



Plot statistics		
Residues in most favoured regions [A, B, L]	196	92.5%
Residues in additional allowed regions |a, b, l, p|	16	7.5%
Residues in generously allowed regions |~a, ~b, ~l, ~p|	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	212	100.0%
Number of end-residues (excl. gly and pro)	2	
Number of glycine residues (shown as triangles)	17	
Number of proline residues	7	
Total number of residues	238	

Fig. 3: Procheck validation result of LipGT. The Ramachandra plot showed that the percentage of the residue that fell within the most favored region was 92.5% whereas the percentage of residue that fell within the additional region was 7.5%

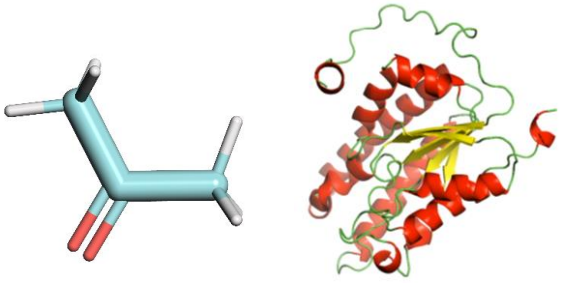
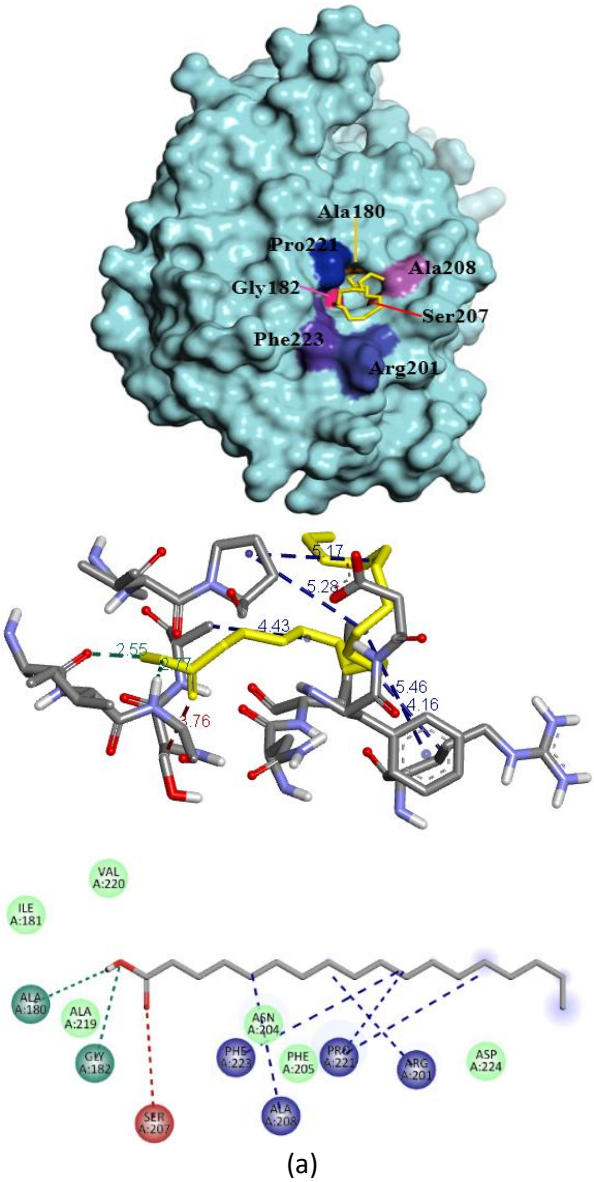


Fig. 4: The tertiary structure of (a). the acetone modeled by Avogadro2 that showed in a stick form. The carbons are cyan in color, the hydrogen atoms are white in color and the oxygen is red in color. (b). The LipGt predicted by Alpha Fold Colab. The α-helices are red in color, β-strands are yellow in color and the loops are green in color

In both dockings, the interactions between the olive oil with the active site of LipGt were predicted using the Biovia discovery studio. The residues involved in the predicted catalytic site were Met 130, Thr 131, Ile132, Tyr 158, Leu 162, Phe 166, Ala 180, Ile 181, Gly 182, Leu 183, Trp 203, Asn 204, Ser 207, Ala 208, Ala 211, Ala 219, Val 220 and Pro 221. The molecular interactions between LipGt and olive oil with and without the acetone are shown in Fig. (5) and Table (1). In the absence of acetone, the olive oil formed 2 hydrogen bonds with Ala 180 and Gly 182, as well as a carbon-hydrogen bond with ser 207. The olive oil also interacted with LipGt through 5 hydrophobic interactions with Pro 221, Arg 201, Ala 208, and Phe 223. Meanwhile, the presence of acetone increased the number of hydrophobic interactions up to 10 with 3 hydrogen bonds. Similar residues interacted with olive oil in the acetone treatment except for Gly182 and Ser207. Besides, more residues such as Ile 132, Tyr 158, and Ala 219 formed hydrogen bonds and hydrophobic interactions with olive oil.



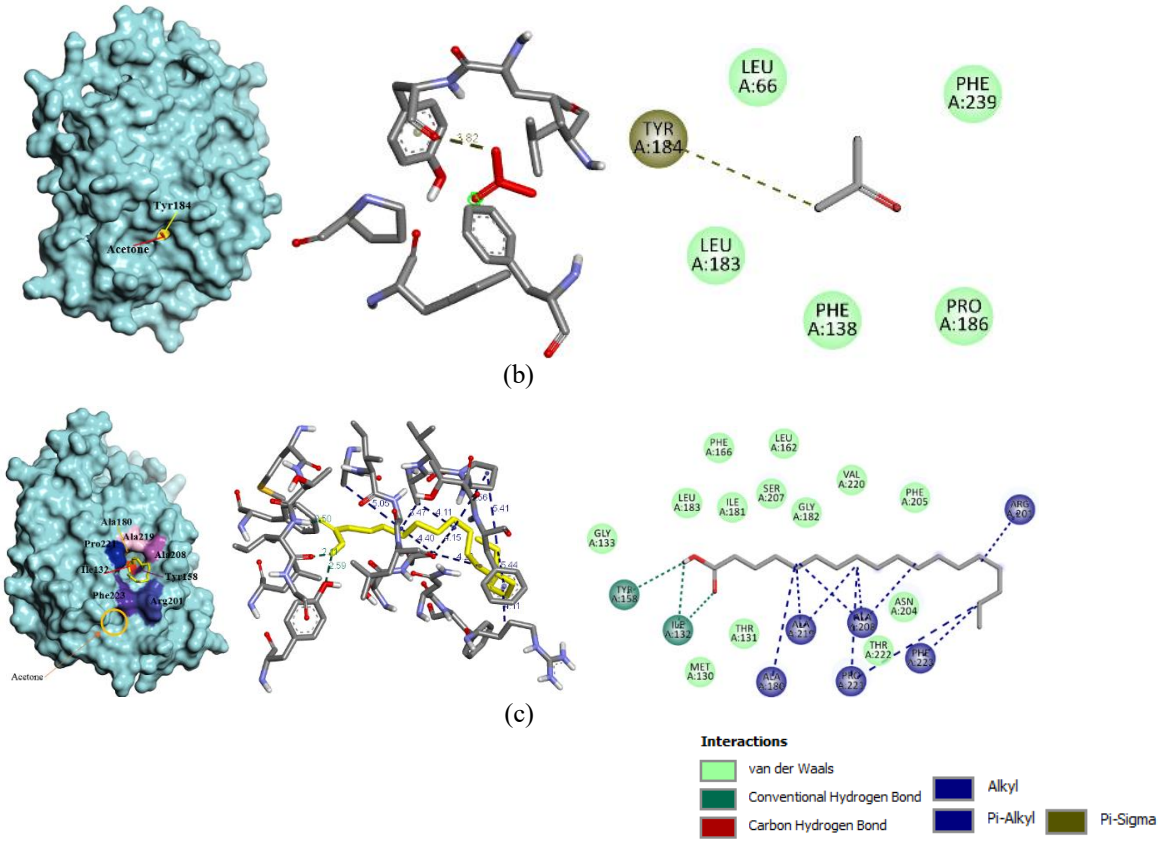


Fig. 5: Molecular surface view of (A) LipGt-olive oil (B) LipGt-acetone (C) LipGt-olive oil-acetone (left panel) (the surface of LipGt is shown in cyan color, the olive oil is shown in yellow stick form while the red stick form of acetone is located below the orange circle surface). 3-2D interaction with hydrogen bonds and other non-bonded interactions (right panel)

Table 1: Molecular docking interaction of Lipase with acetone and olive oil in the presence or absence of acetone

Lipase	Ligand/ substrate	Binding affinity (kcal/mol)	Interaction types	From	To	Distance (Å)
Lipase (absence of acetone)	Olive oil	-4.4	Hydrogen bond	Olive oil	Ala 180	2.55
			Hydrogen bond	Gly 182	Olive oil	2.77
			Carbon hydrogen bond	Ser 207	Olive oil	3.76
			Hydrophobic	Olive oil	Pro 221	5.28
			Hydrophobic	Olive oil	Pro 221	5.17
			Hydrophobic	Arg 201	Olive oil	4.16
			Hydrophobic	Ala 208	Olive oil	4.43
			Hydrophobic	Phe 223	Olive oil	5.46
Lipase	Acetone	-3.4	Hydrophobic	Acetone	Tyr 184	3.82
Lipase	Olive oil	-5.8	Hydrogen bond	Olive oil	Ile 132	2.11
(presence of acetone)			Hydrogen bond	Olive oil	Tyr 158	2.59
			Hydrogen bond	Ile 132	Olive oil	2.50
			Hydrophobic	Olive oil	Pro 221	4.56
			Hydrophobic	Olive oil	Pro 221	5.41
			Hydrophobic	Ala 180	Olive oil	5.05
			Hydrophobic	Arg 201	Olive oil	4.11
			Hydrophobic	Ala 208	Olive oil	4.15
			Hydrophobic	Ala 208	Olive oil	4.23
			Hydrophobic	Ala 208	Olive oil	4.40
			Hydrophobic	Ala 219	Olive oil	4.11
			Hydrophobic	Ala 219	Olive oil	3.47
			Hydrophobic	Phe 223	Olive oil	5.44

Discussion

Lipase isolated from *Geobacillus thermodenitrificans* (LipGt) is a valuable biocatalyst as it has a greater tolerance against the organic solvent and a higher thermostability. In the experiment, the LipGt was cloned, harvested, and purified through several steps, improving the quality of the LipGt for the following studies. Based on our findings, the activity of LipGt significantly increased in the presence of 25%(v/v) acetone after incubation at 65ºC. A similar result was reported by Anuradha *et al.* (2013). The lipase bacillus sp. HT 19 can tolerate a broad range of organic solvents and showed increased activity after incubation with 50% (v/v) of the solvents at 70°C for 1 h. The organic solvents included butyl-alcohol (309.23%), hexane (135.88%), and acetone (115.03%). Meanwhile, DMSO and benzene can completely suppress the lipase activity (Jiang and Xiumeng, 2017). Nevertheless, lipase 2 from *Geobacillus thermocatenulatus* (BTL2) was inhibited by 1% (v/v) acetone and its activity decreased to 82.29% after incubation at pH 8.0 and 60°C for 1 h. However, its activity was enhanced by ethanol (107.29%), n-butanol (102.93%), and n-hexane (117.90%) (Zhang *et al*., 2020). Although all the lipases stated above are isolated from thermophiles, they have different tolerance towards various organic solvents with different concentrations.

The kinetic study is important because it allows a better understanding of catalysis reactions and enzyme modifications to improve their activities. In the presence of acetone, the values of K_m, V_{max}, and K_{cat} for the LipGt assay were increased. However, the data obtained contradicted the fundamental concept of the Michaelis–Menten equation as the K_m and V_{max} increased at the same time. The study discovered by Liu *et al.* (2012) stated that the enzyme activity can be enhanced by the organic solvent with increased K_m and V_{max} values. The presence of the 2% tetrahydrofuran increased the flexibility of polygalacturonase and its modified structure, altering the microenvironment of the catalytic site to allow easier binding of the substrate. Therefore, the activity of polygalacturonase and its modified structure improved when their K_m and V_{max} values were increased. Nevertheless, the findings of Md. Zahid *et al.* (2013) stated differently as the increments of K_m and K_{cat} by organic solvents demonstrated an increase in reaction rate but a decrease in lipase affinity. The increased K_{cat} was caused by the enhancement of the substrate hydrolysis rate once it was bound to the enzyme. Different enzymes isolated from different organisms have distinct properties and tolerance against the organic solvents. Since the LipGt activity was increased in the acetone, it was proposed that the enzyme affinity towards substrate and reaction rate could be increased although the kinetic parameters increased in one direction.

After removing the signal peptide, the quality of the deduced mature LipGt structure was improved. It had a 92.5% of Ramachandran plot which exceeded 90% and all the residues fell within the most favoured regions as well as the additionally allowed regions. It depicted that the deduced LipGt had a good stereochemical quality. The deduced mature LipGt also passed the analysis of ERRAT (98.6%) and verify3d (81.9%). It indicated that the overall quality of the deduced mature LipGt was good and it can be used for the following molecular docking process. Based on the result, the binding affinity of olive oil with LipGt improved in the presence of acetone as it increased the total interacted residues and the number of hydrophobic interactions at the catalytic site. Hydrophobic interactions are significant in maintaining the tertiary structure of the protein. They can increase the resistance and stability of protein at elevated temperatures and thus the unfolding rate becomes slower (Xu *et al*., 2020). According to the study of Patil *et al*. (2010), the increased hydrophobic interactions at the catalytic site can improve the binding affinity of the enzyme. It was suggested that the interaction between acetone with LipGt increased the core hydrophobicity and thus improved the binding affinity of olive oil towards LipGt.

The acetone can enhance and prolong the activity of LipGt after 24 h of incubation at a higher temperature. The increased hydrophobic interaction at the core active site of LipGt by acetone enhanced the resistance towards the higher temperature denaturation, maintaining its folding. At higher temperatures, the lipase catalysis became faster as the kinetic energy of the substrate also increased. Besides, the increased hydrophobicity influenced the enzyme affinity towards the substrate, improving the catalytic efficiency of LipGt. It was believed that acetone performed as an activator to improve the activity and binding affinity of LipGt.

The effect of organic solvent on lipase activity was worth investigating as lipases are one of the major biocatalysts used in industrial areas. In the presence of organic solvents, lipase can catalyze the transesterification to produce biodiesel which can help to provide a sustainable energy resource (Ugur *et al*., 2014). Thus, the kinetics and molecular studies of the solvent-tolerated, thermostable LipGt discovered in the present work could be employed as guidelines in the biodiesel manufacturing and organic synthesis industries. Since both lipase and acetone can be utilized as detergent, thereafter the acetone and lipase could be mixed in a detergent to facilitate the reaction and remove the strains easily. Although acetone can improve the activity of LipGt, it is a harmful chemical substance that can cause negative effects on human health when exposed or ingested in a higher concentration, for example, acetone exposure resulted in coma, ataxia, and cardiovascular collapse (Umeh *et al*., 2021). Thus, it limits the

utilization of LipGt with acetone in the industry fields like the food industry.

The lipase activity is directly proportional to the concentration of organic solvent until a limit. If exceeding the limit, the lipase activity will be inhibited by the organic solvent. Thus, further studies are recommended to determine the limits of acetone concentration in the LipGt assay. Nevertheless, the interactions between acetone and LipGt cannot be identified in detail through protein docking simulation studies. Therefore, it is advised that molecular dynamics simulations be performed in the future to analyze these interactions more thoroughly.

Conclusion

Different lipases isolated from different organisms have distinct organic solvent tolerance. The LipGt activity was significantly affected by acetone, in which acetone altered its active site microenvironment, enhanced its binding affinity towards the substrate, and subsequently increased its catalytic efficiency. The findings imply that the LipGt had a greater tolerance toward acetone and its activity can be enhanced in the acetone treatment. Thereafter, LipGt could be utilized as a potential biocatalyst with acetone in the biotechnology industry such as biodiesel and detergent industries, improving the yield and reducing the cost.

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Author's Contributions

Ammar Khazaal Kadhim Almansoori and Lo Hui Yu: Conceptualized, designed the research idea, and analyzed data. Conducted the experiments, wrote the main manuscript, reviewed and edited. Provided the analytical tools of *in silico* experiments, analyzed data, reviewed, and edited.

Alyaa Abdulhussein Alsaedi: Conducted the experiments, authored the main manuscript, and participated in the review and editing process.

Rashidah Abdul Rahim: Provided resources, and contributed to the review and editing of the manuscript.

Ethics

This article is original content that hasn't been published before. The corresponding author (RAR) attests that all authors have reviewed and approved the works. This article does not contain any studies with human participants or animals performed by any of the authors. The research complies with ethical guidelines and standards.

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