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The Effect of H133V of α-Amylase *Bacillus licheniformis* F11 in High Temperature using Molecular Dynamics Simulation

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Catalyst plays a central role in the chemical industry. In the starch processing industry, acid-based catalyst is used to degrade starch. Unfortunately, this catalyst is not environmentally friendly. Enzymes catalyzing the starch degradation are the alternative biological catalyst that reduces the potential risks caused by a conventional catalyst. Bacillus licheniformis α-amylase F11 (BLA F11), a locally sourced enzyme from Indonesia, has a great catalytic activity but is less tolerant at high temperatures is promising to be used. Ideally, industrial hydrolysis requires an enzyme with relatively stable at extreme temperatures (95-105˚C). To reach this condition, modification of the BLA F11 at the structural level is required to improve the thermal stability. Substitution at position 133 from valine to histidine was applied to the structure. This modification is expected to be stable at high temperatures. This study aimed to determine the effect of H133V on the structure of BLA F11 using molecular dynamics simulation. In this study, the structure of BLA F11 and its mutant was built using homology modeling and the structural behavior in high temperature was observed using molecular dynamic simulation. Bacillus licheniformis α-amylase (PDB ID: 1OB0) was used as a positive control because of thermostable. The results showed that the modification of H133V was predicted to have more hydrophobic interactions, which are associated with the positive control.
This mutation also reduced the flexibility of the nearby loop and improved the secondary structure stability of β-sheet in the B domain which involved thermal stability.

Keywords: Molecular dynamics simulation | Bacillus licheniformis | thermostability | amylase

Catalyst is one of the important properties used in industry, particularly in the starch processing industry. To break down the starch, this industry usually used acid catalysts such as hydrochloric acid and sulfonic acid. However, using this catalyst has the potential to harm the environment and the organism (1). Hence, using enzymes as a substitute has several advantages, such as being environmentally friendly producing high product conversion (2).

α-Amylase produces oligosaccharides by cleaving the α-1,4-glycosidic bond at amylopectin and amylose chain in starch (2). α-Amylase derived from microorganisms is favorable to industrial application because of a vast quantity, low-cost production, and accessibility of genetic modification (3). Bacteria that produced α-amylase commonly sourced from *Bacillus* sp., such as *B. clause, B. calculus, B. amyloliquefaciens, B. substilis,* and *B. licheniformis* (4). Protease, α-amylase, and lipase are among the enzymes secreted by *B. licheniformis* (5). Locally source α-amylase from *B. licheniformis* F11 (BLA F11) had been isolated from shrimps' shells waste at Palembang, Indonesia. This enzyme has a high catalytic activity, but it is less temperature tolerant. On the other hand, amylolytic enzymes with great thermostability are more valuable in industrial application.

The liquefaction stage of starch hydrolysis requires an enzyme that acts at high temperatures to break down the starch granule that occurs at 95-105˚C in a pH of 6.0 - 6.5 for 90 minutes (6). The ability to keep the conformational shape through the salt bridge conformation, intermolecular interaction, and a low-flexibility amino acid composition are some of the elements that influence the enzyme's stability (7). The thermostability analysis of an enzyme is closely related to the structure of the enzyme. One of the potential methods for designing a modified enzyme with desired properties is Computer-Aided Enzyme Design (CAED) (8). Some studies have used this method to save time and cost consuming before recombinant protein experiments, such as modification of *Pseudoalteromonas haloplanktis* α-amylase to improve thermostability (7) and the use of molecular dynamics simulation to improve substrate adsorption of *Saccharomycopsis fibuigera* R64 α-amylase (9).

Modification of BLA F11 sequence can be applied to improve the thermostability through molecular dynamics (MD) simulation. Machius et al. studied the thermostability of BLA by introducting hydrophobic interactions on the enzyme's surface. The result on five substitution amino acid revealed an increase in enzyme's stability at 85^0C at pH 5.6 for 7.5 hours, compared to 14 minutes at 85^0C for the native (10). As a result, increasing the hydrophobic interactions on

the BLA structure is expected to stiffen the structure, resulting in increased stability.

In this study, the modification of BLA F11 sequence was applied at H131V, which change the histidine at 133 to valine. The homology modeling method was used to build the model structure of BLA F11 and its mutant, and MD simulation at high temperature was used to the behavior of structural dynamics. The structural stability of the wild-type and its mutant was compared to that of a the positive control. It is hoped that results would be useful to the development of BLA F11 for industrial purposes.

Methods

Equipment

All procedures were carried out on an Ubuntu-powered computer 20.04.2.0 LTS and equipped with an Intel Xeon ® CPU E5-2678 v3 @2.5 GHz × 24, 16 GB RAM, and a GPU NVIDIA Ge Force RTX 2080Ti 6 GB,

Material

The sequence of BLA F11 was retrieved from https://www.ncbi.nlm.nih.gov, with the accession number CAJ70707.1. The structure of BLA with PDB code 1OB0.

Modeling of BLA F11 and Mutant

The model structure of BLA F11 was constructed by homology modeling approach using MODELLER 9.23(11). Pfam analysis (http://pfam.xfam.org/) was used to determine the functional region of BLA F11 sequence(12). The template for homology modeling was selected based on the highest identity percentage score, folding pattern, and also the template structure quality. The Discrete Optimized Protein Energy (DOPE) score and GA341 were used to choose a high-quality model. The quality of model structure was assess by Ramachandran plot using PROCHECK server, VERIFY-3D method, and Z-score using ProSA-web. A sililar method was used to model the mutant of BLA F11. Substitution amino acid was selected from histidine to valine at position 133 (PDB code 1OB0 numbering scheme). Two calcium ions (Ca^{2+}) and sodium ion (Na^{+}) were added to both models in the A/B domain and another Ca^{2+} placed in the A/C domain, respectively, as cofactors. The structure of BLA with PDB code 1OB0 was selected as a positive control since it stable at high temperature (85˚C) (10).

Molecular Dynamics Simulation

All systems were prepared by using pdb4amber in AMBER18 including the cysteine-type and the protonation state of histidine at specific chemical environments. The TIP3P box water molecule model was added to the system with the shortest distance of 10 Å between protein and the edge of the box. Chloride ions were added to neutralize the system. AMBER18 was used for all the minimizations. The first minimization was calculated by the steepest descent algorithm for 1000 steps, followed by 2000 steps of a conjugate gradient with 500 kcal/mol$Å^2$ of harmonic restraint were applied for the backbone atoms. A final 1000 steps minimization was performed by an unrestrained conjugate gradient to remove any steric clashes among the atoms. The system was gradually heated to 373 K (100˚C) with NVT ensemble using harmonic restraint of 5 kcal/mol$Å^2$ on the backbone atoms. The restrained are being released gradually by 1 kcal/mol$Å^2$ until it reaches 0 for 1000ps of NPT equilibration. Then, the production stage was run for 100 ns with the time step of 2 fs in NPT ensemble with all hydrogen atom constrained using the SHAKE algorithm. The pressure was controlled using Berendsen barostat with the coupling constant of 1ps and the target pressure of 1 bar, whereas the temperature was controlled by the Langevin thermostat with a collision frequency of 1 ps^{-1}. The nonbonded cutoff value was used at 9 Å and the long-range electrostatics was treated using the Particle Mesh Ewald.

Analysis of Molecular Dynamics Simulation

The trajectories of MD were analyzed using cpptraj module in AmberTools18. The root-mean-square deviation (RMSD) was calculated to observe the deviation between the original and the simulated structure. Whereas the fluctuation of the amino acid residues was calculated by the root-mean-square fluctuation (RMSF). Molecular interaction was performed at the mutation point to evaluate the structural stability. While the visual inspections were performed using VMD (13).

Results

Modeling of BLA F11

The sequence of BLA F11 consists of 512 amino acids. In this model, the 31 first sequences were not modeled because the template did not cover this structure, and it is not a functional region according to Pfam analysis (Suppl 1) (12). BLA F11's structure, like the general structure of α-amylase, was predicted to be made up of two domains: a catalytic domain (A/B domain) and an unknown domain (C domain). The model was constructed with the template of PDB code 1OB0 because they shared a high sequence identity with the percentage of 98.34%, a satisfactory resolution at 1.83 Å, and a similar folding pattern identified by the Phyre server (http://www.sbg.bio.ic.ac.uk/) (Suppl 2). The residual alignment of BLA F11 with the template is shown in Fig. 1. There are eight residues found different in BLA F11 compared to 1OB0 which is located in the A/B domain. The constructed model from homology modeling needs to be assessed preventing template misfolding from wild-type conformation. The assessment model was evaluated by DOPE and GA341 values. The lowest DOPE score of BLA F11 was obtained in -5913 and the GA341 value was 1. The lower the DOPE score, the more stable the model. Whereas the value of GA341 more than 0.7 represents a good quality folding pattern (11). The DOPE profile of the model and template showed that they had a similar pattern (Suppl 3).

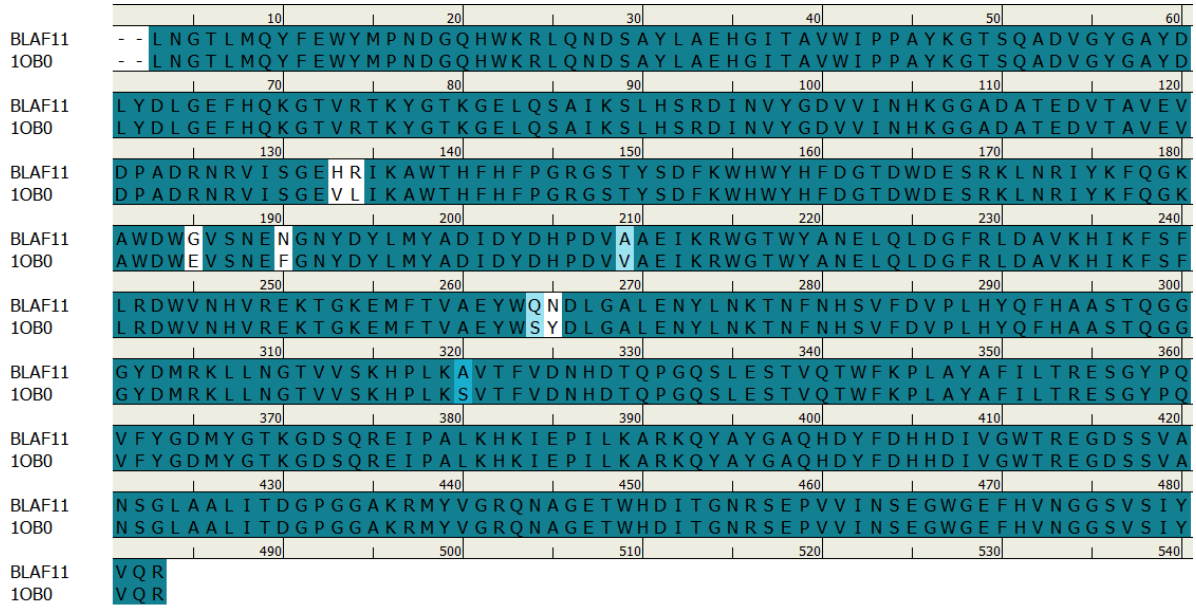


Figure 1 Sequence alignment of BLA F11 and template (PDB 1OB0).

The Ramachandran plot, VERIFY-3D, and ProSA were used to evaluate the quality of BLA F11 model. It was shown that BLA F11 model has 91.1% residues found in the allowed region and none in the disallowed region (Fig. 2A). In general, 90% of residues located in allowed regions are classified as a good quality model (14, 15). Furthermore, the compatibility model coordinated with its sequence is verified by VERIFY-3D giving the score 97.35% residues have averaged 3D-1D score more than 0.2, regardless it is derived from X-Ray, NMR, or computational method. A minimum criterion of 80% of the amino acids scores more than 0.2 indicating a good accuracy model (16). Also, the z-score analysis provides an overall accuracy quality model in the X-Ray region with a score of -10.11 (Fig. 2B) (17). From these evaluations, we can assume that our model meets a good quality structure. When both structures were superimposed and compared to the positive control, the overall structure revealed that each enzyme has a comparable folding structure, with an RMSD value of 0.203. (Fig. 3).

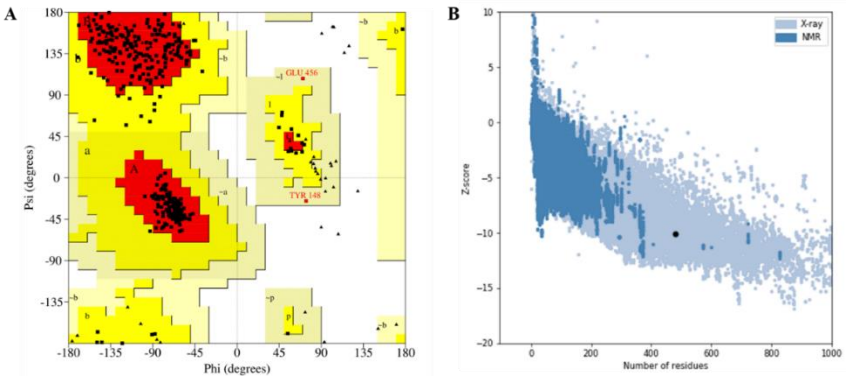


Figure 2 Model evaluation assessed by Ramachandran plot (A) and Z-score analysis (B).

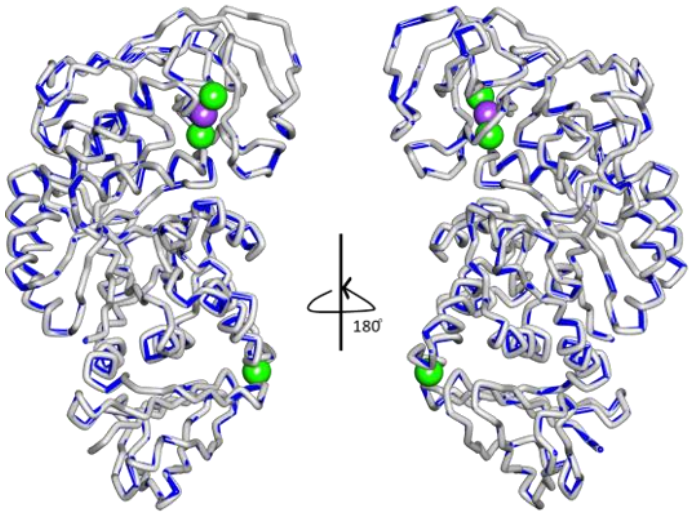


Figure 3 The overlay structure of the BLA F11 model and its template, which are visualized in blue and grey, respectively. The calcium ion and sodium ion are drawn in the green ball and purple ball, respectively.

Molecular Dynamics Simulation

MD simulations were performed in 100 ns to observe the behavior of positive control, BLA F11, and BLA F11MT. The overall stability of all structures was assessed by root-mean-square deviation (RMSD) analysis which calculates the deviation of backbone atoms of the protein referred to initial structure's backbone atoms, averaged over backbone atoms (18). A continuous increase in RMSDs value from the initial structure indicated a labile conformation or instability model and also offers the perception of tertiary structure (19–21). The RMSD profile of the positive control showed a constant profile in the average value of 1.23 Å through 100 ns simulation which considered good global stability. While the BLA F11 and its mutant have average RMSD value greater than the positive control, 1.64 and 1.58 Å, respectively (Fig. 4A). The positive control and wild-type had a similar profile for 60 ns and increased up to 2 Å until the end of the simulation. Otherwise, the modification of H133V on BLA F11MT leading overall a constant profile during the simulation although a sharped peak occurs in 20 ns. This phenomenon occurred due to the flexibility of loop 370 to 377 in domain A and it might be due to the absence of substrate which changed all catalytic sites to be closed conformation (14,20,21). Nevertheless, this structure can restabilize so that the deviation could be decreased at the end of the simulation.

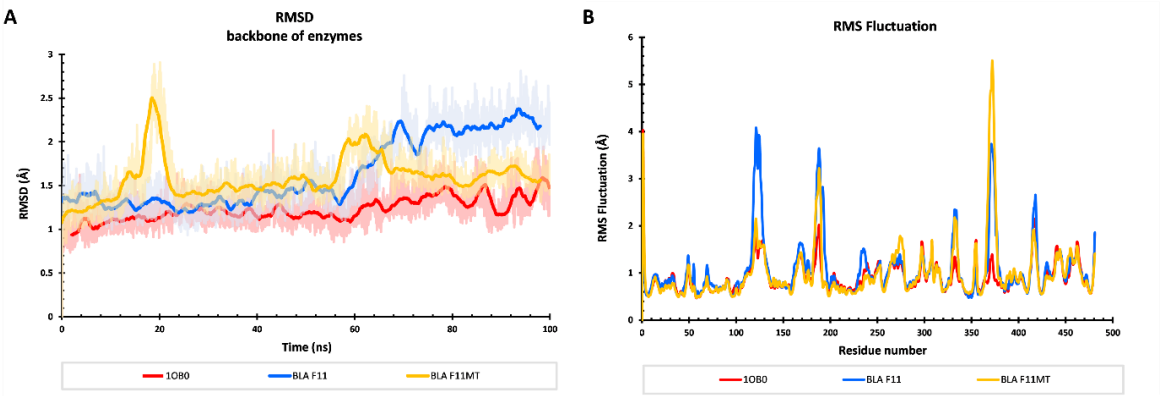


Figure 4 RMSD and RMSF profile throughout 100 ns simulation. A) RMSD of protein backbone which presented in 200 frames moving average. B) Residual RMSF profile of all systems.

The residual fluctuation of all systems was calculated by the root-mean-square fluctuation (RMSF) analysis. The high fluctuation indicates flexible amino acid which can change the structure to the unfolded state. The residues with lower RMSF values indicate a stable and rigid structure (20,21). As shown in Fig. 4B the high fluctuation (>2 Å) was observed at N-terminal because this region was not restrained so that it could move freely. Another high fluctuation was observed in domain A/B of wild-type and mutant (domain B in 118-129, domain A in 184 to 193, and 370 to 377). The high fluctuation in domain B was located on the surface structure. This region has two-fold higher fluctuation in wild-type compared to the positive control. It was due to the lacked hydrophobic interaction. As expected, modification of H133V could decrease the fluctuation in which equal to the positive control. Residue 133 is located near the loop 118 to 129 which can stabilize the nearby loop and decrease the fluctuation value. High fluctuations in domain A (184 to 193) were due to their natural loop flexibility where they lie at the end of the central cleft of domain A and B with the side chain fully solvent-exposed also forms a cage of metal ions (22). The last sharped peak (residue 370 to 377) is located near

catalytic sites, it was due to the absence of substrate and the lack of interaction that stabilizes its loop.

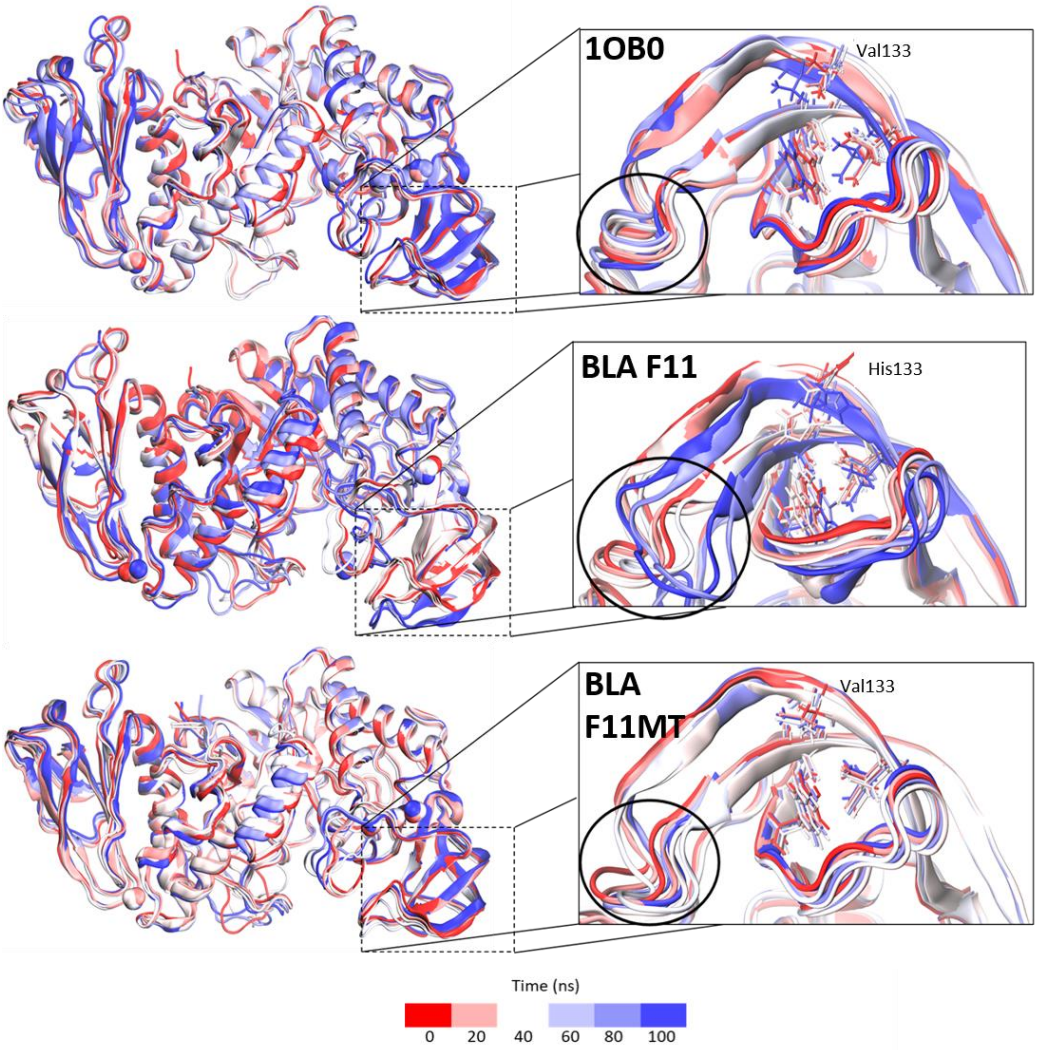


Figure 5 Time evolution snapshot every 20 ns at loop B-domain BLA, BLA F11, and BLA F11MT. The differences between them are highlighted in a black circle, a loop 118 to 129.

The time evolution snapshot calculated every 20 ns showed that the loop at B-domain is more fluctuating in BLA F11 than its mutant (Fig. 5). This observation was due to the mutation at position 133. The stabilize of B-domain related to the stabilize structure at high temperature, therefore the more stable the B-domain the more stable structure at high temperature, it implies that the selection of the substitution amino acid in this region is crucial (23,24). A new hydrophobic interaction occurs at BLA F11MT between Val133 and Tyr175, and this interaction also occurs in positive control. Contrarily, this interaction is missing in the wild-type. Another hydrophobic network has occurred between Ile135, Tyr175, and Ala117. This interaction is essential to stabilize the loop at the B-domain. Another important intramolecular interaction is hydrogen bond interaction which contributes to protein folding, stability, and function (18). The global energy structure of BLA F11MT calculated by MMGBSA method was more negative than others, indicating this mutant still stable (Fig. 6). Other analysis to evaluate the stability of BLA F11MT was the secondary structure evolution through simulation (Fig. 7). It showed that the structure relatively stable and there were not change. Further experiment is needed to investigate these results. Also, another condition that mimics the real system is required to investigate the behavior of enzymes. This study should offer valuable insight to rationalize the design of BLA F11MT before the recombinant protein experiment in the lab.

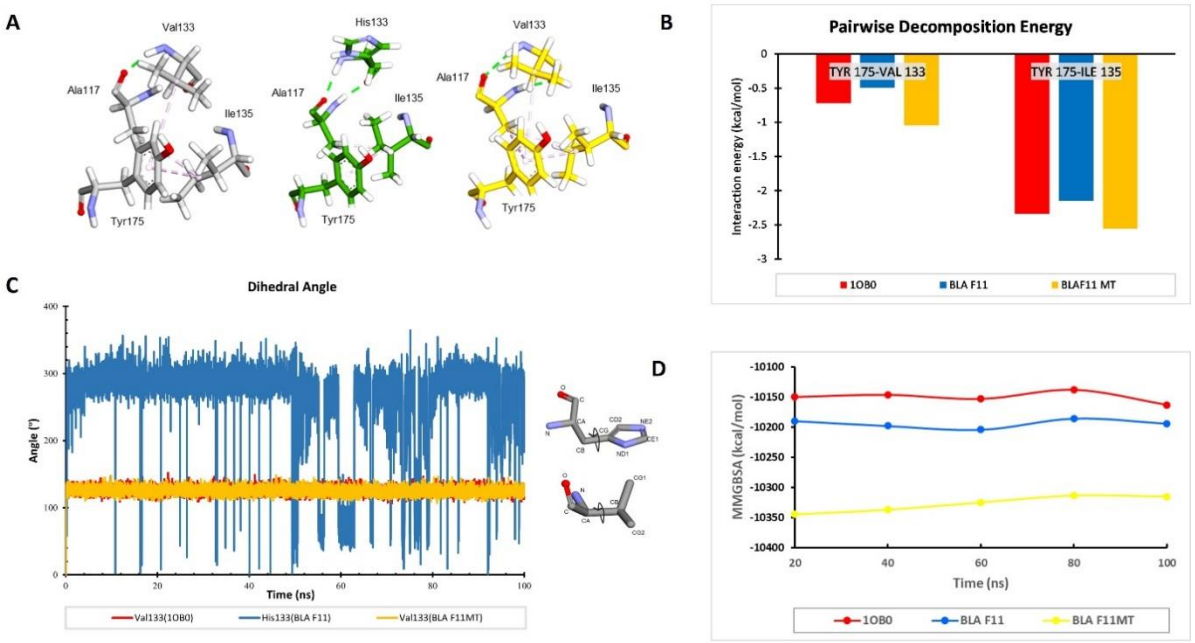


Figure 6 A) The interaction analysis around 133 of BLA, BLA F11, and BLA F11MT, represented in grey, green and yellow colors, respectively. The green-dashed-line and purple-dashed line indicated hydrogen bond and hydrophobic interaction. B) Pairwise decomposition of the interaction energy between 175 and 173 also 175 and 135. C) Dihedral analysis of residue 133. D) Global energy calculated by MMGBSA method.

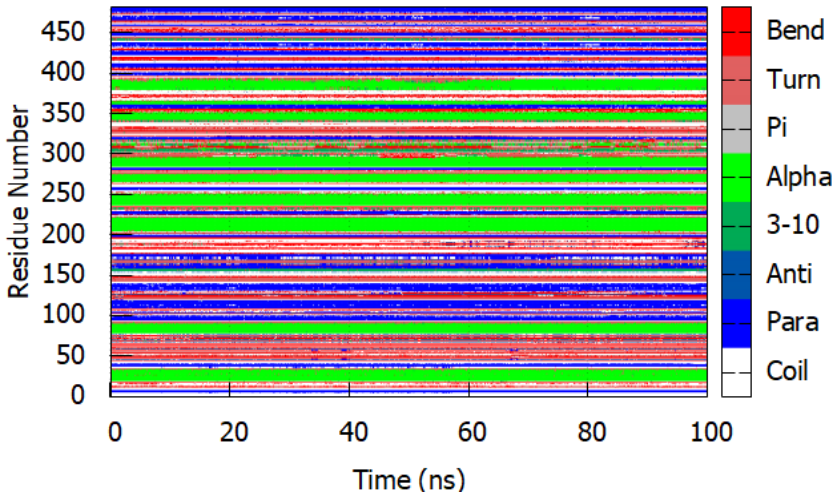


Figure 7 Secondary structure evolution throughout 100 ns simulation of BLA F11MT

Discussion

Over the recent decade, some investigations of mutational analysis on BLA have been conducted in order to identify the manipulation of BLA or to improve its thermal stability. Declerck et al. (25) analyzed the sequences of three amylases from BLA, BAA (*Bacillus amyloliquefaciens*), and BSTA (*Bacillus stearothermophilus*) and discover that BLA had excess histidine residues which somehow could contribute to improving the thermostability. One of the critical positions is His133, where substituting an amino acid on this side can either stabilize or destabilize the thermostability in comparison to the wild-type (26). The crystal structure of the free-calcium ion form of BLA has been used to describe the effect of this mutation (23). Declerck et al. (23) investigated about half of the possible amino acid residue had stabilizing effect compared to original histidine. The results showed that valine is a good candidate to replace histidine in this position. Stabilizing substitution of His133 must compensate for the loss of hydrogen bond. This condition could be achieved by van der Waals contacts with underlying hydrophobic contacts. A good correlation was found between protein stability and β-sheet propensity in which residue 133 lies in the β-sheet region. In fact, His133 is the first residue of the β-strand. Breaking the polar connections to nearby residues of the initial residue of α-helix or β-strand is the most energy-consuming step in protein unfolding. Therefore, it is expected that terminal positions between secondary structure-forming propensities of α-helix or β-strand are helpful to structure stability (27). Substitution H133V also improved close packing by reducing the number of internal cavities through the hydrophobic package. Additionally, H133V was chosen

because histidine is more polar than valine, which means that histidine could form a hydrogen bond with water and could damage the structure of the enzyme (10).

The mutation at position 133 formed hydrogen bonds interaction with Ala117 at all enzymes with equal occupancy. This observation was in accordance with the pairwise decomposition energy. It was shown that the interaction between Tyr175 and Val133 more negative in BLA F11MT and also the interaction between Tyr175 and Ile135. Dihedral angle analysis was also observed to evaluate the stability of the mutant. This analysis is calculated when 4 atoms formed an angle during simulation (14). It is shown that valine is stable over 100 ns both in mutant or positive control. Otherwise, histidine is relatively unstable although it has a cyclic ring in its structure.

Conclusion

This study was aimed to determine the effect of H133V on the structure of BLA F11 using molecular dynamics simulation. Although BLA F11 and 1OB0 share a high identity, the characteristic of both enzymes is different especially in high temperature. The substitution point at position 133, from histidine to valine was successfully improved the structural stability in MD simulation which was conducted at high temperature. Nevertheless, our findings should be validated in other experiments. This sequence can be introduce to other microorganisms, bacteria or yeast, and produce the protein through DNA recombinant.

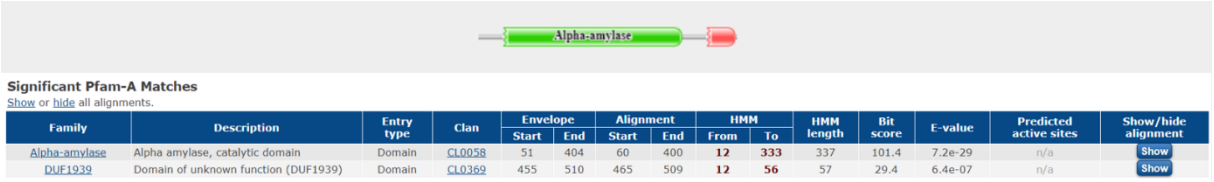
References

1. Damhus T, Kaasgard S, Olsen HS. Enzymes at work. Fourth. Denmark: Novozyme; 2013. 1–76 p.
2. Hua X, Yang R. Enzymes in Starch Processing. In 2015. p. 139–69.
3. Gopinath SCB, Anbu P, Arshad MKM, Lakshmipriya T, Voon CH, Hashim U, et al. Biotechnological Processes in Microbial Amylase Production. Biomed Res Int. 2017;2017.
4. Pavithra S, Ramesh R, Aarthy M, Ayyadurai N, Gowthaman MK, Kamini NR. Starchy substrates for production and characterization of Bacillus subtilis amylase and its efficacy in detergent and breadmaking formulations. Starch/Staerke. 2014;66(11–12):976–84.
5. Niyonzima FN, More SS. Concomitant production of detergent compatible enzymes by Bacillus flexus XJU-1. Brazilian J Microbiol. 2014;45(3):903–10.
6. Azmi AS, Malek MIA, Puad NIM. A review on acid and enzymatic hydrolyses of sago starch. Int Food Res J. 2017;24(March):265–73.
7. Lim SJ, Hazwani-Oslan SN, Oslan SN. Purification and characterisation of thermostable $α$-amylases from microbial sources. BioResources. 2020;15(1):2005–29.
8. Gupta M, Wadhwa G, Sharma SK, Jain CK. Homology modeling and validation of SAS2271 transcriptional regulator of AraC family in Staphylococcus aureus. Asian Pacific J Trop Dis. 2013;3(1):1–4.
9. Baroroh U, Yusuf M, Rachman SD, Ishmayana S, Hasan K, Subroto T. Molecular dynamics study to improve the substrate adsorption of Saccharomycopsis fibuligera R64 alpha-amylase by designing a new surface binding site. Adv Appl Bioinforma Chem. 2019;12:1–13.
10. Machius M, Declerck N, Huber R, Wiegand G. Kinetic stabilization of Bacillus licheniformis α-amylase through introduction of hydrophobic residues at the surface. J Biol Chem. 2003 Mar;278(13):11546–53.
11. Fiser A, Šali A. MODELLER: Generation and Refinement of Homology-Based Protein Structure Models. Methods Enzymol. 2003;374:461–91.
12. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, et al. The Pfam protein families database: Towards a more sustainable future. Nucleic Acids Res. 2016;44(D1):D279–85.
13. Humphrey W, Dalke A, Schulten K. VMD : Visual Molecular Dynamics. J Mol Graph. 1996;14:33–8.
14. Yusuf M, Baroroh U, Hasan K, Diana S, Ishmayana S, Subroto T. Computational Model of the Effect of a Surface-Binding Site on the Saccharomycopsis fibuligera R64 α -Amylase to the Substrate Adsorption. Bioinform Biol Insights. 2017;11:1–8.
15. Laskowski R, MacArthur M, Moss D, Thornton J. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of applied crystallography. J Appl Crystalography. 1993;26(2):283–91.
16. Eisenberg D, Lüthy R, Bowie JU. VERIFY3D: Assessment of protein models with three-dimensional profiles. Methods Enzymol. 1997;277(April):396–404.
17. Baroroh U, Yusuf M, Rachman SD, Ishmayana S, Syamsunarno RAA, Levita J, et al. The Importance of Surface-Binding Site towards Starch-Adsorptivity Level in ? -Amylase : A Review on Structural Point of View.
18. Du J, Dong J, Du S, Zhang K, Yu J, Hu S, et al. Understanding Thermostability Factors of Barley Limit Dextrinase by Molecular Dynamics Simulations. Front Mol Biosci. 2020;7(April):1–11.
19. Maulana FA, Ambarsari L, Wahyudi ST. Homology modeling and structural dynamics of the glucose oxidase. Indones J Chem. 2020;20(1):43–53.
20. Housaindokht MR, Monhemi H, Hosseini HE, Sadeghi Googheri MS, Najafabadi RI, Ashraf N, et al. It is explored that ionic liquids can be suitable solvents for nitrile hydratase catalyzed reactions: A gift of the molecular modeling for the industry. J Mol Liq. 2013;187:30–42.
21. Janati-Fard F, Housaindokht MR, Monhemi H. Investigation of structural stability and enzymatic activity of glucose oxidase and its subunits. J Mol Catal B Enzym. 2016;134(September):16–24.
22. Machius M, Declerck N, Huber R, Wiegand G. Kinetic Stabilization of Bacillus Licheniformis Alpha-Amylase Through Introduction of Hydrophobic Residues at the Surface. JBiolChem. 2003;278:11546.
23. Declerck N, Joyet P, Trosset JY, Garnier J, Gaillardin C. Hyperthermostable mutants of Bacillus licheniformis α-amylase: Multiple amino acid replacements and molecular modelling. Protein Eng Des Sel. 1995 Oct;8(10):1029–37.
24. Joyet P, Guérineau M, Heslot H. Cloning of a thermostable α-amylase gene from Bacillus licheniformis and its expression in Escherichia coli and Bacillus subtilis. FEMS Microbiol Lett. 1984 Mar;21(3):353–8.
25. Declerck N, Machius M, Joyet P, Wiegand G, Huber R, Gaillardin C. Hyperthermostabilization of Bacillus licheniformis α-amylase and modulation of its stability over a 50°C temperature range [Internet]. Vol. 16, Protein Engineering, Design and Selection. 2003. p. 287–93. Available from: https://academic.oup.com/peds/article-lookup/doi/10.1093/proeng/gzg032
26. Joyet P, Declerck N, Gaillardin C. Hyperthermostable variants of a highly thermostable alpha-amylase. Bio/Technology. 1992;10(12):1579–83.
27. Declerck N, Machius M, Chambert R, Wiegand G.

Hyperthermostable mutants of Bacillus licheniformis α-amylase: thermodynamic studies and structural interpretation His133Tyr and Ala209Val (named therein H133Y and A209V. Vol. 10, Protein Engineering. 1997.

Supplementary data

Supplementary 1

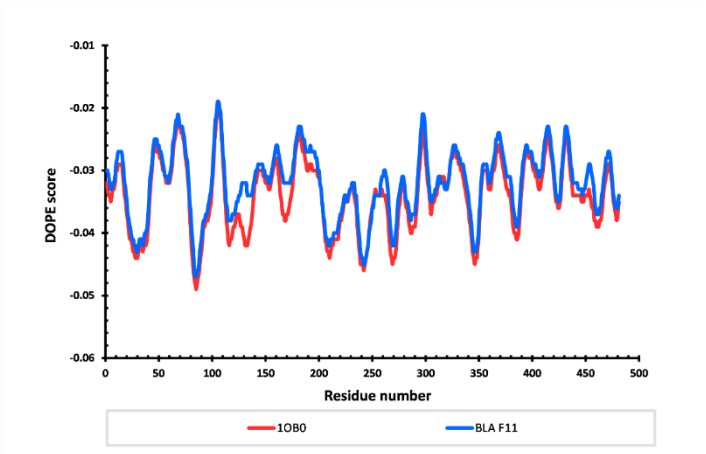


Pfam analysis of *Bacillus licheniformis F11* sequence

Supplementary 2

Phyre analysis of *Bacillus licheniformis F11* sequence

Supplementary 3



DOPE score analysis of BLA F11 model and template

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