A protein engineering to create the high thermostable formate dehydrogenase enzyme for biocatalysis applications

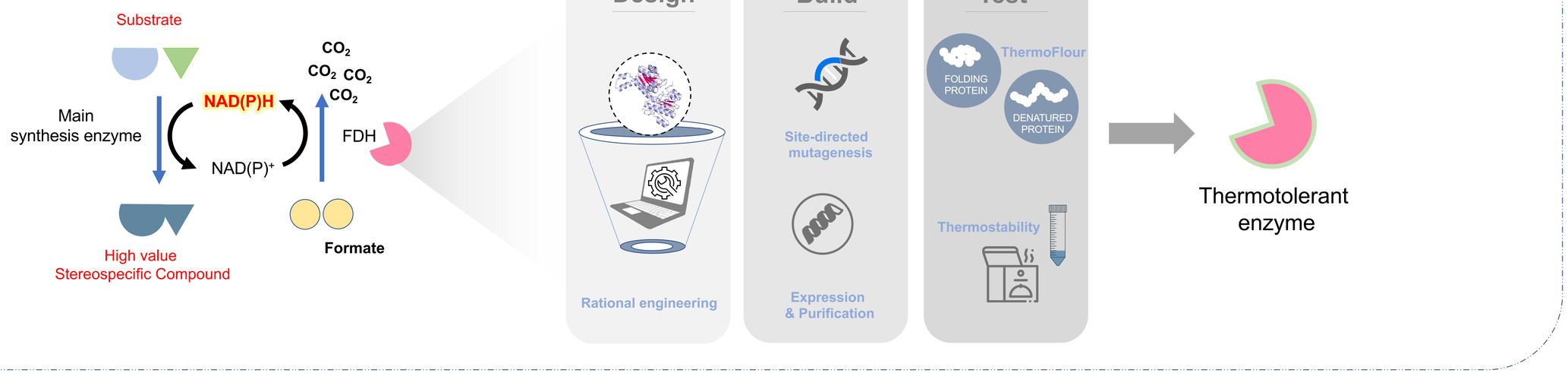
Rattima Boonkumkrong[1], Paweenapon Chunthaboon[1], Ruchanok Tinikul[2]

[1]Student of Department of Biochemistry and Center of Excellence in Protein and Enzyme Technology (CPET), Faculty of Science, Mahidol University, Bangkok 10400, Thailand

[2] Assistant Professor, Department of Biochemistry and Center of Excellence in Protein and Enzyme Technology (CPET), Faculty of Science, Mahidol University, Bangkok 10400, Thailand

***Email:** rattima2014@gmail.com, rattima.boo@student.mahidol.edu

GRAPHICAL ABSTRACT



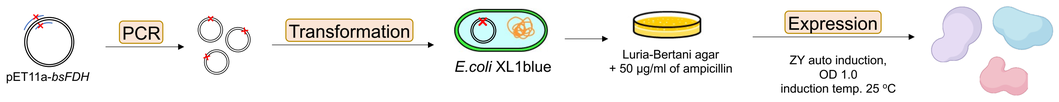
INTRODUCTION & AIM

FDH is one of the most appropriate enzyme to be used as a co-enzyme regenerating system for biocatalysis. Finding new FDH with high catalytic efficiency would be beneficial. Previously, our research team has characterized a novel FDH enzyme from *Bacillus simplex* (BsFDH). The enzyme has high activity, providing 4.4-fold greater specific activity than that of FDH from *Pseudomonas* sp101 (PsFDH), a commercial FDH used in real industry. Unfortunately, the pain-point of low thermostability of this enzyme still hampers its robustness. Therefore, this research project aims to engineer the BsFDH enzyme to improve its thermostability by using a rational design strategy.

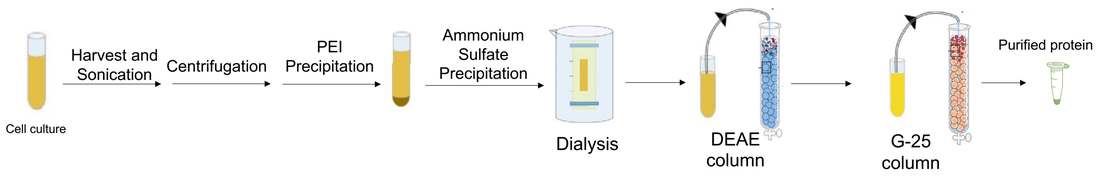
EXPERIMENTS

1. Computational prediction

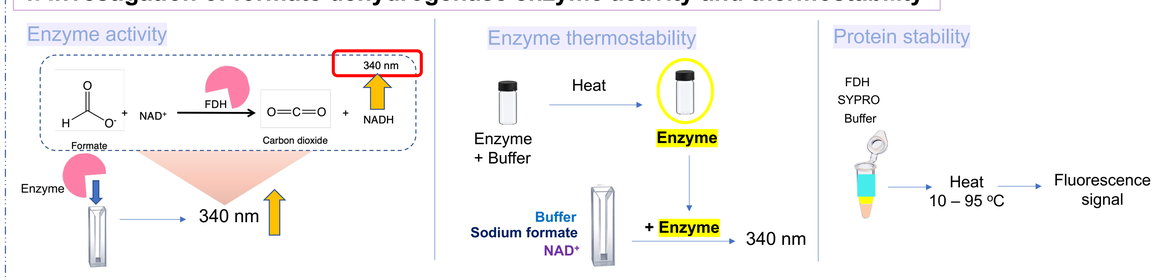
2. Construction and expression of BsFDH wild-type and mutant enzymes



3. Purification of wild-type and mutant FDH enzymes



4. Investigation of formate dehydrogenase enzyme activity and thermostability



RESULTS

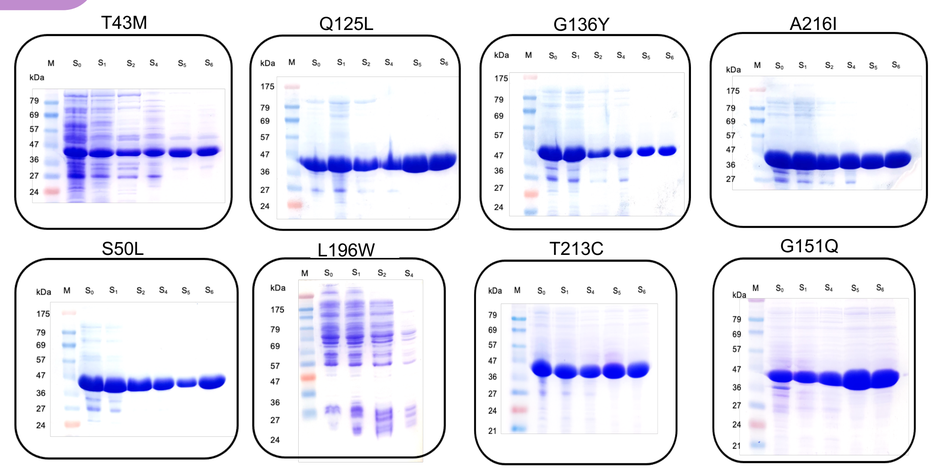


Figure 1 SDS-PAGE analysis of BsFDH mutants in each purification step A 20 µg protein from each purification step was examined using 12% SDS-PAGE . Lane M; protein marker, Lane S_0; crude extract, Lane S_1; protein after precipitated by 0.5% (w/v) polyethyleneimine, Lane S_2; protein after precipitated by 40-60% ammonium sulfate, Lane S_4; protein after dialysis, Lane S_5; protein after purified by DEAE-Sepharose column, Lane S_6; protein after Sephadex-G25 gel filtration. The enzymes showed overexpression bands with an approximate size of around 38.5 kDa and obtained more than 95% purity judged by SDS-PAGE analysis.

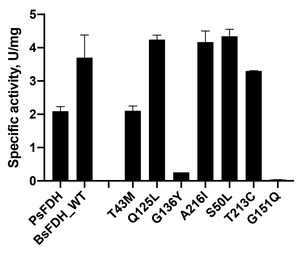


Figure 2 The specific activity of wild-type and mutant BsFDHs. The FDH activity was measured based on the formation of NADH. The assay reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 and consisted of 0.4 mM NAD^+, 800 mM sodium formate and 0.25 µM FDH. The reaction was monitored at a wavelength of 340 nm at 25 °C.

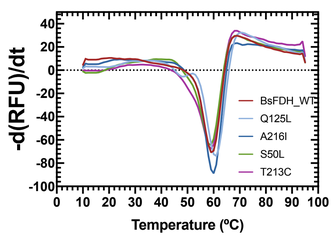


Figure 3 The melting curves of BsFDH wild-type and mutants. (A) The melting curves are presented by plotting the first derivative of the fluorescence emission as a function of temperature. The T_m corresponds to the lowest point of the first derivative graph and the assay was performed in triplicate.

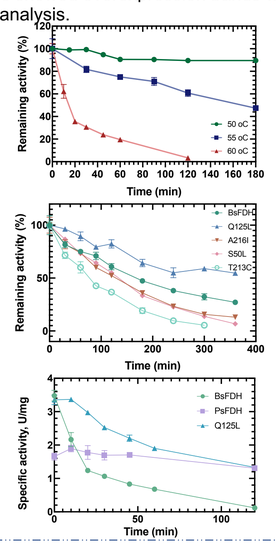


Figure 4 Thermostability assay. (A) Thermal inactivation of wild-type BsFDH at different incubation temperatures.(B) Thermostability assay at 55 ºC of wild - type and BsFDH variants. Percent remaining activities were calculated relative to the activity at time 0 and the assay was carried out in triplicate. (C) Thermostability assay at 60ºC and the specific activities are presented.

DISCUSSION AND CONCLUSION

This study used *in silico* approaches to rationally design variants of BsFDH with improved thermostability. Although more than 60 target variants were suggested by the program. We implemented three rational criteria for selecting candidate variants based on knowledge of enzyme structure and function to reduce the number of candidates to be studied. The results showed the thermostability improvement of Q125L variant. The Gln125 position is located near to the dimeric interface, where the residues 4 Å-around Gln125 position in protein structure are mostly hydrophobic amino acids. The changing of Gln to Leu, which possesses hydrophobic property, may support the hydrophobic interaction, leading to improve the thermostability of the Q125L variant. In addition, Q125 may be a potential position for further improvement of BsFDH thermostability by site-saturation mutagenesis.

ACKNOWLEDGEMMENTS

The authors acknowledge Development and Promotion of Science and Technology Talents Project (DPST) (to RB), and a Research Grant for New Scholar (Office of the Ministry of Higher Education, Science, Research and Innovation, Grant number: RGNS 63-179) (to RN), Faculty of Science, Mahidol University, Department of Biochemistry and Center of Excellence in Protein and Enzyme Technology (CPET), Faculty of Science, Mahidol University for the technical support.