DEVELOPMENT OF HIGHLY ACTIVATED PLASTIC DEGRADING ENZYME

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Keywords:Plastic Degrading Enzyme, Artificial variant, plastic environmental issues, Industrial enzyme

1. Background and Aim of Study

The serine esterase cutinase is a plastic-degrading enzyme belonging to the α/β hydrolase superfamily. This enzyme is secreted by microorganisms such as fungi to hydrolyze ester bonds. Cutinase catalyzes the hydrolysis of several polymers including PET (polyethylene terephthalate).

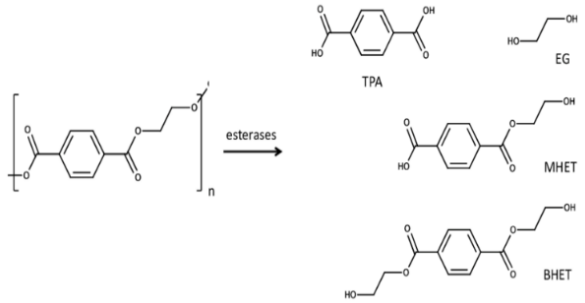


Figure1: Pet depolymerization

Cutinase (Cut190) from saccharomonospora viridis AHK190 has 3 Ca^{2+} binding sites. Enzymatic activities are activated when Ca^{2+} binds to the functional site 1 of cutinase.

The aim of this study is to prepare a cutinase variant that degrades plastic wastes with high efficiency and develop a photo-switching mechanism to establish a biodegrading system with the enzyme for industrial application.

In this study, I focus on Cut190 to prepare its highly activated variant. Because cutinase other than other plastic degrading enzymes is relatively smaller and can easily utilize the E. coli expression system. Subsequently, The Phenylalanine 77 which is located in the key region of the enzymatic function is substituted by the bulkier amino acid Tryptophan to induce activation of esterase activity.

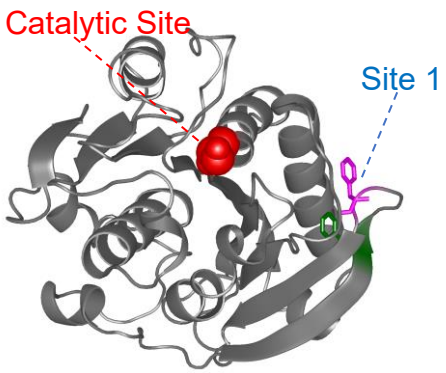


Figure2: Cut190 was purified with a Co2+-NTA column.

2. Materials and Methods

Expression and purification of Cut190

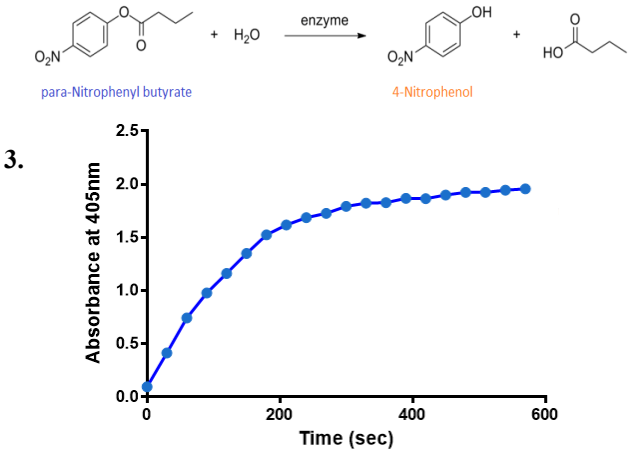
Cut190 DNA was incorporated into the pET15b vector. Cut190 expression plasmids were transformed into Escherichia coli Rosetta2 (pLysE). Cut190 was purified with a Co2+-NTA column.

Esterase assay.

Esterase assay was performed using the synthesized substrate analog, para-nitrophenyl butyrate. The reaction of hydrolysis para-nitrophenyl butyrate is

accompanied by an increase in absorbance at the wavelength 405 nm and a colour change from colourless to yellow as shown in Figure 4. The concentration of 4-Nitrophenol (4-NP) produced could then be calculated with the Beer-Lambert Law, as the extinction coefficient of 4-NP at 405 nm is 18,000 M-1 cm-1.

Figure 3: hydrolysis reaction of para-Nitrophenyl butyrate by esterase and monitoring its product 4-NP at 405nm.



3. RESULTS and DISCUSSION

3-1. Purification of Cut190 and its mutant P77W

The Cut190 expressed by E.coli was purified a using a cobalt chelate column. SDS-PAGE results showed a band around the predicted molecular weight of 28,400 for Cut190.

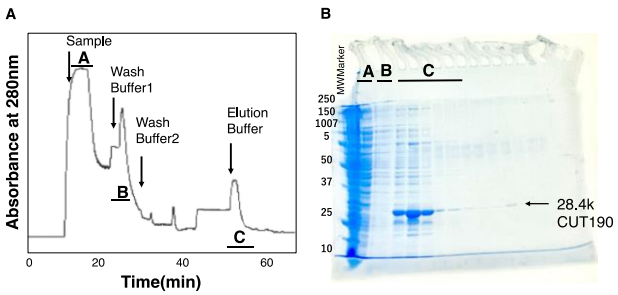


Figure 4: Elution profile of Cut190 and SDS-PAGE

Cut190 mutant P77W was prepared using primer DNA and mutation kit and its expression by E. coli was observed.

3.-2. Esterase assay of Cut190

The esterase activity of the purified Cut190 was measured in the presence and absence of calcium by measuring absorbance at 405 nm. As shown in Figure 5, the purified Cut190 exhibited almost the same esterase activity in the presence and absence of calcium.

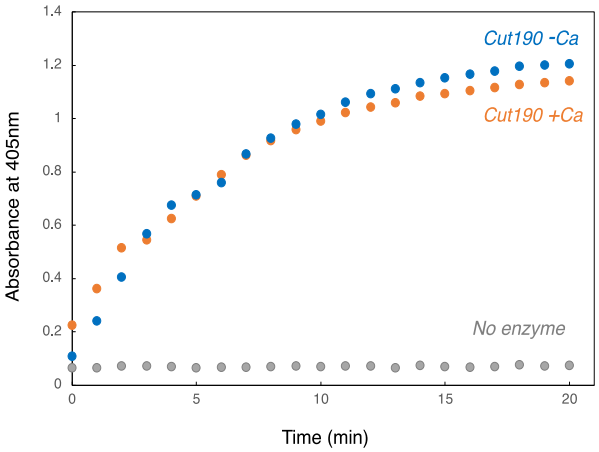


Figure 5: Time course of hydrolysis reaction of para-Nitrophenyl butyrate by Cut190 in the presence and absence of calcium.

4. CONCLUSION

We have successfully expressed and purified Cut190 and confirmed its enzymatic activity in the presence and absence of Ca^{2+}. Cut190 mutant P77W was also expressed by E.coli expression system.

5. Reference

1) Calado, C.R.C.; Monteiro, S.M.S.; Cabral, J.M.S.; Fonseca, L.P. Effect of pre-fermentation on the production of cutinase by a recombinant *Saccharomyces cerevisiae J. Biosc. Bioeng.*, 93, 354-359, 2002.

6. Future Plane

1. To compare the esterase activity of Cut190 with its mutant P77W.
2. Incorporation of photochromic azobenzene derivatives into the functional site of Cut190.
3. Photo-control of esterase activity of Cut190 modified by azobenzene derivative.