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Characterization of *Ideonella sakaiensis* mono-(2-hydroxyethyl) terephthalate hydrolase (MHETase) and Optimization of Reaction Conditions to Improve Plastic-Degrading Activity
การศึกษาสมบัติของเอนไซม์โมโน-2-ไฮดรอกซีเอทิลเทเรฟทาเลตไฮโดรเลส จากเชื้อแบคทีเรีย *Ideonella sakaiensis* และสภาวะที่เหมาะสมของปฏิกิริยาที่เพื่อการย่อยสลายพลาสติก

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ABSTRACT

Ideonella sakaiensis MHETase catalyzes the hydrolysis of mono-(2-hydroxyethyl) terephthalate (MHET) as part of the PET degradation pathway. This work reports recombinant expression and preliminary characterization of MHETase W397A, which has been shown to exhibit higher activity than other MHETase variants (Gottfried J. Palm, 2019). MHETase W397A was expressed in *Escherichia coli* and purified by immobilized metal affinity chromatography. The ability of the enzyme to hydrolyze MHET at different pH was then assessed. It was found that MHET hydrolysis activity of MHETase W397A was highest at pH 7. Next, the effects of temperature, ionic strength, organic solvents, and detergents on the activity of MHETase W397A will be studied.

บทคัดย่อ

โมโน-2-ไฮดรอกซีเอทิลเทเรฟทาเลตไฮโดรเลส (MHETase) เร่งปฏิกิริยาการย่อยสลายสารโมโน-2-ไฮดรอกซีเอทิลเทเรฟทาเลต (MHET) ในวิถีการสลายพลาสติกพอลิเอทิลีน เทเรฟทาเลต (PET) ในงานวิจัยนี้คณะผู้วิจัยได้ทำการผลิตเอนไซม์ MHETase ที่มีกลายกลายพันธุ์ W397A ซึ่งมีการรายงานว่าสามารถเร่งปฏิกิริยาได้ดีกว่าเอนไซม์ดั้งเดิม และเอนไซม์พันธุ์กลายอื่น ๆ ที่ทำการศึกษา (Gottfried J. Palm, 2019) โดยใช้เทคนิครีคอมบิแนนท์ดีเอ็นเอ และทำการแสดงออกใน *Escherichia coli* จากนั้นจึงนำเอนไซม์ที่ทำบริสุทธิ์แล้วไปศึกษาสมบัติการทำงาน จากการศึกษานี้พบว่าเอนไซม์ MHETase ที่มีกลายกลายพันธุ์ W397A สามารถทำงานได้ดีที่สุดที่ pH 7 ในขั้นตอนต่อไปคณะผู้วิจัยจะทำการศึกษาผลของอุณหภูมิและความเข้มข้นของไอออน ตลอดจนศึกษาความสามารถของ MHETase W397A ในการทนต่อตัวทำละลายอินทรีย์และสารซักล้างต่าง ๆ

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Introduction

Polyethylene terephthalate (PET) is the most widely used synthetic polymers in food and beverage packaging industry due to its strong gas barriers and flexible properties. The increase of PET consumption in the world has become a significant problem to the environment because PET has a very long lifetime ranging from 16 to 48 years (Müller, 2005). PET is produced by polymerization of terephthalate (TPA) and ethylene glycol (EG). Current recycling methods can cover only a fraction of PET. In 2015, It was estimated that only 20 percent of global plastic waste was recycled (Roser, 2018). Most PET recycling employs a mechanical process which consumes energy and typically generate downgraded product (Grigore, 2017). Another available method in PET recycling is chemical recycling, which employs chemical processes for plastic depolymerization. Chemical recycling needs harsh conditions, including high temperature and extreme pH. While it is possible to chemically degrade PET to its basic building blocks that can be used in PET re-synthesis. The chemical processes need active and selective catalysts that are expensive and are energy-intensive (Garcial J.M., 2017). In addition, both mechanical and chemical recycling processes are not entirely environmentally friendly as they require the use of toxic volatile organic compounds that can harm plant and animal lives (d'Ambri'eres, 2019). Therefore, eco-friendly strategies are needed for plastic waste management.

Recently, a PET-degrading bacterium *Ideonella sakaiensis* was discovered that was able to grow on PET film (Shosuke Yoshida, 2016). The bacteria can produce two enzymes– PETase and MHETase – that are used in the conversion of PET to terephthalate and ethylene glycol. First, PETase converts PET to mono-(2-hydroxyethyl) terephthalate (MHET). Then, MHET is converted by the second enzyme, MHETase, to produce terephthalate and ethylene glycol (Shosuke Yoshida, 2016). Both products can serve as carbon and energy source for *I. sakaiensis*. Beneficially, the two products can also be used as building blocks for PET re-synthesis; this make PETase and MHETase attractive for applications in PET recycling. Unfortunately, however, the rates of reactions catalyzed by wild-type enzymes are too slow to be applied in PET recycling. In addition, MHET has been reported to inhibit PETase reaction (Barth M, 2015).

Previous characterization of MHETase enzymes revealed that the W397A MHETase variant, in which Trp397 has been substituted with alanine, had the highest turnover rate among all the studied MHETase variants, including wildtype MHETase (Gottfried J. Palm, 2019). However, the optimal reaction conditions for MHET hydrolysis, such as pH, temperature, and ionic strength have not been thoroughly studied. In addition, the ability of MHETase to catalyze the reaction in the presence of organic solvents and detergents have not been investigated.

Here, recombinant expression and preliminary characterization of *Ideonella sakaiensis* MHETase W397A is reported. The protein was heterologously expressed in *Escherichia coli* and purified by immobilized metal affinity chromatography (IMAC). The ability of the enzyme to hydrolyze MHET at different pH was assessed using high performance liquid chromatography (HPLC). Within the pH range

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tested (pH 6-8), it was found that rate of MHET hydrolysis by MHETase W397A variant was highest at pH 7. Next, the effects of reaction temperature and ionic strength on the rate of MHET hydrolysis by MHETase W397A, as well as the tolerance of the enzyme to organic solvents and detergents will be assessed.

Understanding the optimal conditions for MHET hydrolysis can improve the enzyme activity and enhance enzyme stability, which will benefit future efforts in enzyme engineering for plastic waste degradation.

Objective of the study

The aim of the study is to recombinantly express and purify *Idenoella sakaiensis* MHETase W397A variant and to optimize the pH for MHET hydrolysis reaction of *I. sakaiensis* MHETase W397A variant.

Methodology

1. Expression and purification of MHETase W397A variant

The gene encoding MHETase W397A was codon optimized and synthesized by GenScript, USA, then cloned into pCOLDII vector using NdeI and HindIII restriction sites. The construct encodes residues 20–603 of *I. sakaiensis* MHETase W397A, with a N-terminal His6-tag tag and a TEV protease cleavage site. To express the MHETase W397A variant, the recombinant plasmid was transformed into SHuffle® T7 Express Competent *E. coli* cells (New England Biolabs) and selected on Luria-Bertani (LB) agar plates containing 100 μg/mL ampicillin. A single colony was picked into 100 ml of LB media containing 100 μg/mL ampicillin and grown overnight at 30 °C, 250 rpm. For protein expression, 2.5 L baffled Erlenmeyer flasks containing 1 L medium supplemented with 100 μg/mL ampicillin was inoculated with 10 ml of the overnight starter culture. The cells were grown at 33 °C, 160 rpm until the optical density at 600 nm (OD_{600}) reached 1.0, at which point isopropyl β-D-1thiogalactopyranoside (IPTG) was added to the final concentration of 1 mM to induce protein expression. When OD_{600} reached 2.5, the temperature was shifted down to 16 °C, and the overexpression was allowed to proceed overnight. Cell samples were collected at 1, 2, 4 and 16 hours after OD_{600} reached 2.5 to study the soluble and insoluble expression. The cells were harvested by centrifugation at 4 °C, 10,000 × g for 20 min and stored at −20 °C.

The cells pellet was resuspended in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM imidazole and 1 mM dithiothreitol (DTT), and sonicated. The lysate was centrifuged at 17, 000 × g, 4 °C for 10 min. The soluble and insoluble fractions, as well as the whole cell lysate was then analyzed by SDS-PAGE. Since MHETase W397A variant was found to express in the insoluble form, lysis buffer was then supplemented with various additives to solubilize the enzyme (Leibly, 2012). The additives (0.5 M mannitol, 0.5 M, L- proline, 0.375 M L-arginine, 1 M xylitol, 0.5% (w/v) cetyl trimethylammonium bromide

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(CTAB), 10% (w/v) sarkosyl, 0.5% (w/v) Triton X-100 were added individually in the lysis buffer, which was used for cell resuspension and sonication in small scale (1 ml).

Once candidate additives that could solubilize MHETase W397A had been identified (0.05% (w/v) CTAB and 0.5% (w/v) sarkosyl), they were tested in large-scale (1 L) cell lysis and protein purification. For MHETase purification, the clarified cell lysate was loaded on a gravity flow column with Ni-NTA agarose, washed with lysis buffer supplemented with 20 mM imidazole and eluted with lysis buffer supplemented with 250 mM imidazole. In this experiment, sarkosyl was the only additive that could successfully solubilize MHETase W397A in large scale. Therefore, the concentration of sarkosyl was then varied between 0.5% to 1% (w/v) to determine the optimal concentration for MHETase W397A solubilization. Ni-NTA elution fractions containing MHETase W397A were combined and concentrated using Amicon® Ultra 15 centrifugal filter unit (Merck-Millipore) with a molecular weight cut-off of 50 kDa, then dialyzed against 50 mM Tris-HCl, pH 7.5, 150 mM NaCl. The concentration of MHETase was assessed by using absorbance 280 nm and the molar extinction coefficients of 98,320 M^{-1} cm^{-1} as calculated from ProtParam analysis (Gasteiger E., 2005). From the calculation, the final concentrations of MHETase were 4.24 µM. The protein was supplemented with 10% (v/v) glycerol and stored in -80 ^{o}C.

2. Activity Testing

MHET hydrolysis activity of MHETase W397A was determined using MHET that is generated from Bis (2-hydroxyethyl) terephthalate (BHET) hydrolysis by PETase as substrate and analyzed using high performance liquid chromatography (HPLC).

BHET (10 mM) was incubated with either 1 µM PETase alone or a combination of 1 µM PETase and 0.5 µM of MHETase in 50 mM Tris-HCl, pH 8.0, 20% (v/v) of dimethyl sulfoxide (DMSO) solution at 30 ^{o}C for 2 hours. To stop the reaction, 5 mM of phenylmethylsulphonyl fluoride (PMSF) was added to the reaction and incubated at room temperature for 20 min. Then, the solution was diluted 10 times with 50 mM Tris-HCl buffer at pH 8.0 and filtered through a 0.22-micron syringe filter. After that, compound BHET, MHET, and TPA from the reaction were monitored by HPLC.

For HPLC analysis, C_{18} column (5 µm, 4.6 x 150 mm) was used. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B was 20% acetonitrile, 0.1% formic acid in water. The flow rate was fixed at 1 ml $\cdot$ min^{-1}, and the absorbance at 240 nm was followed. The gradient used in HPLC was as follows: 0–2 min, 60–80% buffer B, linear gradient; 2–8 min, 80–100% buffer B, linear gradient; 8–16 min, 100% buffer B. BHET and TPA authentic compounds were also analyzed on HPLC as controls.

For pH optimization of MHET hydrolysis reaction, the experiment was set up in two separate steps to ensure that the change in the amounts of TPA generated was due to the effect of reaction condition on the enzyme MHETase W397A, and not on the ability of PETase to hydrolyze BHET. To this end, BHET (10 mM) was first incubated with 1 µM PETase in 50 mM Tris-HCl, pH 8.0, 20% (v/v) of DMSO solution at 30 ^{o}C for 2 hours. The reaction was then stopped by addition of 5 mM of PMSF and incubation

at room temperature for 20 min. The PETase enzyme was remove from the solution by applying the reaction to a centrifugal filter unit (Amicon ® Ultra, Merck Millipore Ltd; 10 kDa cut off), and centrifuged at 4000 x g, 20 min, 4 °C. After PETase has been removed, the filtrate was incubated for 40 minutes for PMSF to completely decay. Then, the solution condition in the flow-through was adjusted to 25 mM Tris-HCl, pH 8.0, 20% (v/v) DMSO, with 50 mM phosphate buffer at pH 6, 7 or 8. Enzyme MHETase (0.2 μM) was added to the solution and incubated at 30 °C for 30 min. PMSF (5 mM) was added to the solution and incubated at room temperature for 20 min to stop the reaction. Control reactions were set up in a similar manner except that the enzyme MHETase was omitted from the reaction. The MHETase and control reactions were diluted 10 times with 50 mM phosphate buffer and filtered through a 0.22-micron syringe filter prior to HPLC analysis. The experiments were performed in triplicates and the concentration of TPA in the reaction was calculated using TPA standard curves prepared at appropriate pH.

For TPA standard curve, the standard TPA solutions at different pH supplemented with 20% (v/v) DMSO were prepared at concentrations of 250, 125, 62.5, 31.25, and 15.6 μm. The calibration curves of each product were constructed from the absorption peak area versus the standard solution concentration.

Results

1. Expression and purification of MHETase W397A variant

To obtain MHETase W397A variant for further characterization, the protein was expressed in SHuffle® T7 Express Competent *E. coli* cells using IPTG induction. Lysis of the overexpressed *E. coli* cells in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM imidazole and 1 mM DTT) revealed that the level of expression was similar at all time points, and that MHETase W397A was expressed in insoluble form, as it was found in the pellet fraction at all time points (Figure 1).
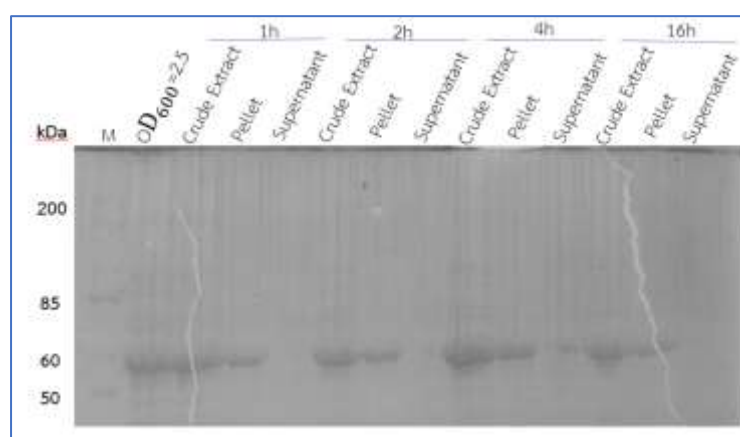

Figure 1: Solubility of MHETase W397A in lysis buffer at different time points. All samples were electrophoresed using 10% SDS-PAGE gel and stained using Coomassie Brilliant Blue. M is protein marker.

In an attempt to solubilize the overexpressed MHETase W397A, the lysis buffer was supplemented with various additives (Leibly, 2012). The seven additives were chosen to be included in our experiment based on the fact that they were among the top performing additives in previous reports in which 144 additives were screened (Leibly, 2012), and were available in our research group. Our results showed that 0.5% (w/v) CTAB or 10 % (w/v) sarkosyl could solubilize the expressed protein in small scale (1 ml) (Figures 2). Further optimization of concentrations of the two additives in the lysis buffer showed that 0.05% (w/v) CTAB or 0.5% (w/v) sarkosyl are the minimal concentration of additive required for MHETase W397A solubilization and serve as good working concentrations in small-scale cell lysis (Figures 3a and 3b).
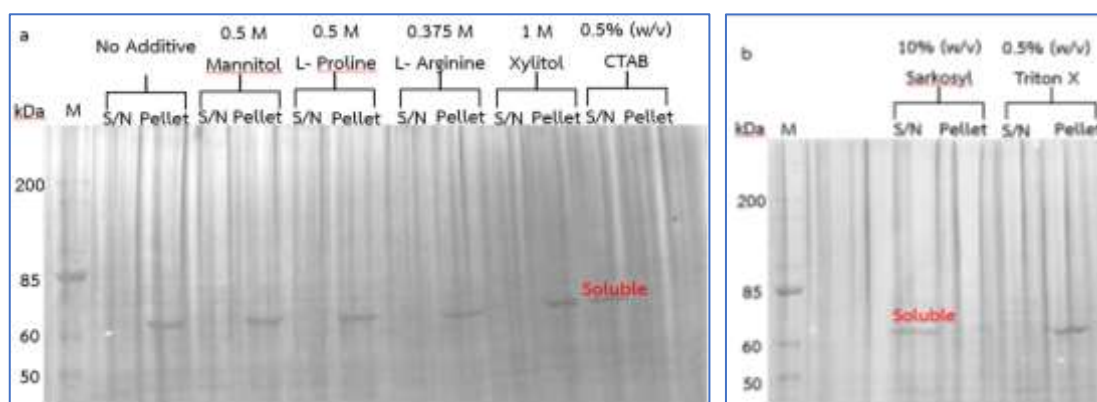

Figure 2: The effects of various additives in lysis buffer on solubility of MHETase W397A. Supplementation with 0.5% (w/v) CTAB and 10% (w/v) sarkosyl were able to solubilize MHETase W397A in small-scale cell lysis (1 ml). All samples were electrophoresed using 10% SDS-PAGE gel and stained using Coomassie Brilliant Blue. M is protein marker. S/N is supernatant.
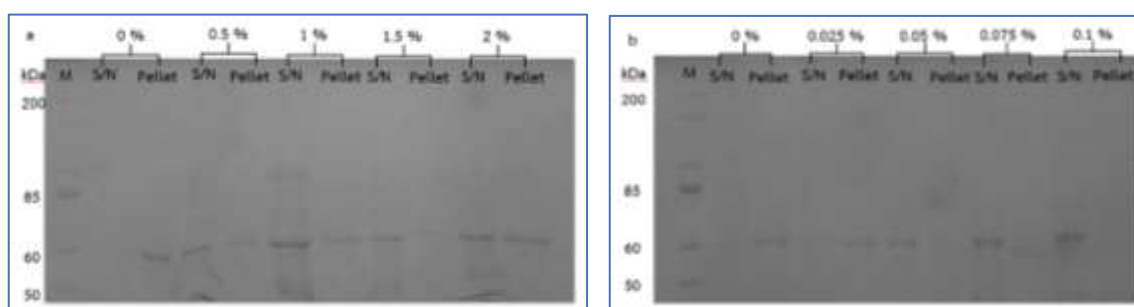

Figure 3: The concentration of sarkosyl (a) and CTAB (b) in the lysis buffer was varied to identify the optimal concentration for solubilization of MHETase W397A expression. Supplementation with 0.5% (w/v) sarkosyl (**a**) or with 0.05% (w/v) CTAB (**b**) was the optimized concentration for MHETase W397A solubilization as judge from small-scale cell lysis. All samples were electrophoresed using 10% SDS-PAGE gel and stained using Coomassie Brilliant Blue. M is protein marker. S/N is supernatant.

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Based on the results of small-scale experiments, large-scale (1 litre) expression and purification of MHETase W397A was performed using 0.5% (w/v) sarkosyl or 0.05% (w/v) CTAB as the additive during cell lysis. The cells were resuspended in lysis buffer with the indicated additive and lysed by sonication. The clarified cell lysate was then subjected to Ni-NTA purification. It was found that 0.5% (w/v) sarkosyl, but not 0.05% (w/v) CTAB, could solubilized MHETase W397A from large-scale expression (Figures 4a and 4b). However, a relatively low level of MHETase W397A was found in the Ni-NTA elution fractions, and a significant amount of protein remained in the insoluble fraction (Figure 4b). Therefore, the concentration of sarkosyl was increased to 1% (w/v) in an attempt to increase the amount of soluble MHETase W397A. The result from this experiment showed that more MHETase W397A could be found in the Ni-NTA elution fraction 1 and 2 (Figure 5) when 1% (w/v) was used during cell lysis. Although a substantial amount of protein remained in the insoluble fraction, the level of protein in the Ni-NTA elution fraction was sufficient for further biochemical characterization. Therefore, the sarkosyl concentration was not increased any further to minimize the possibility of any adverse effects caused by high concentration of sarkosyl on enzyme activity. Ni-NTA elution fractions containing MHETase W397A were combined and concentrated, then dialyzed against 50 mM Tris-HCl, pH 7.5, 150 mM NaCl. The concentration of MHETase was assessed by using absorbance 280 nm and the molar extinction coefficients of 98,320 M^{-1} cm^{-1} as calculated from ProtParam analysis (Gasteiger E., 2005). From the calculation, the final concentrations of MHETase were 4.24 µM. The protein was supplemented with 10% (v/v) glycerol and stored in -80 ^{o}C.
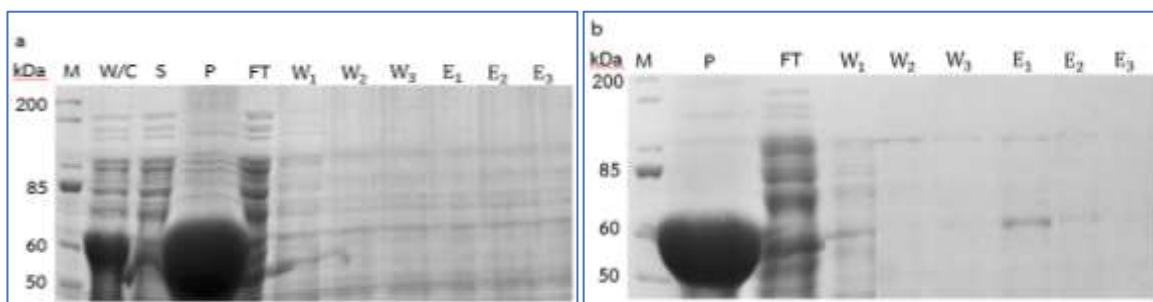

Figure 4: MHETase W397A expression and purification in large scale (1 litre culture). The cells were lysed in the presence of 0.05% (w/v) CTAB (**a**) or 0.5% (w/v) sarkosyl (**b**) and the clarified cell lysate was subjected to purification by Ni-NTA affinity chromatography. While supplementation of lysis buffer with 0.05% (w/v) CTAB could not solubilize MHETase W397A, supplementation with 0.5% (w/v) sarkosyl yielded a low level of MHETase W397A in the Ni-NTA elution fraction. All samples were electrophoresed using 10% SDS-PAGE gel and stained using Coomassie Brilliant Blue. M is protein marker. W/C is whole cell extract. S is supernatant. P is Pellet. FT is flow-through. W_1, W_2, W_3 are washing fractions. E_1, E_2, E_3 are elution fractions.
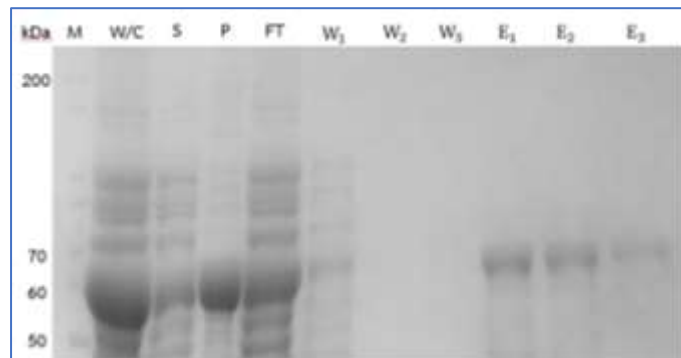
Figure 5: MHETase expression and purification in large scale (1 litre culture) using Ni-NTA column with addition of 1% (w/v) sarkosyl in the lysis buffer. Supplementation with 1% (w/v) sarkosyl was able to give enough protein for further biochemical protein characterization. All samples were electrophoresed using 10% SDS-PAGE gel and stained using Coomassie Brilliant Blue. M is protein marker. W/C is whole cell extract. S is supernatant. P is Pellet. FT is flow through. W_1, W_2, W_3 are washing fractions. E_1, E_2, E_3 are elution fractions.

2. Activity Testing

To assess the activity of MHETase W397A variant, the MHET substrate is required. As MHET is not commercially available, it was obtained from the hydrolysis of BHET by PETase enzyme. BHET (10 mM) was incubated with either PETase alone or a combination of PETase and MHETase at 30 $^{\circ}$C for 2 hours. The reaction was then stopped by addition of 5 mM PMSF and incubated at room temperature for 20 min. Subsequently, the reaction products were analyzed by HPLC. Consistent with a previous study (Makoto Furukawa, 2018), incubation of BHET with PETase alone yielded MHET as the major product, with a small amount of TPA (Figure 6a). Inclusion of MHETase in the reaction resulted in complete conversion of BHET and MHET to TPA (Figure 6b). BHET compound without added enzyme served as a control (Figure 6c).
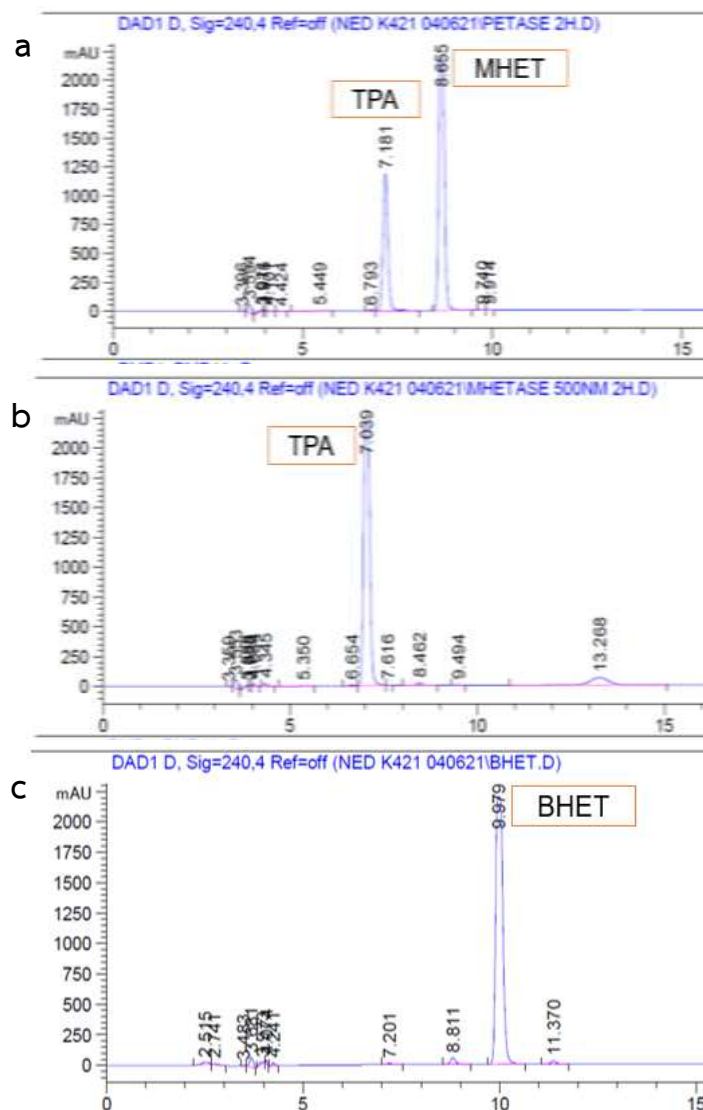
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Figure 6: MHETase could completely convert MHET to TPA. (**a**) Incubation of 10 mM BHET with 1 μM PETase yielded MHET as the major product and TPA as the minor product. (**b**) Incubation of 10 mM BHET with both enzymes (1 μM PETase and 0.5 μM MHETase) resulted in complete conversion of BHET to TPA. The reactions were incubated at 30 °C for 2 hours and analyzed by HPLC at wavelength 240 nm. BHET compound without added enzyme served as control (c). mAU is milli-absorbance units.

Next, as part of the effort to determine optimal reaction conditions for MHET hydrolysis by MHETase, the MHET hydrolysis reaction were set up at varying pH using sodium phosphate buffer pH 6–8. Phosphate buffer was chosen based on its ability to function as a buffer over the neutral pH range, and the pH range of 6–8 was selected based on a previous study, which reported that wild-type MHETase

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performed well under these conditions (Gottfried J. Palm, 2019). The experiment was set up in two separate steps to ensure that the change in the amounts of TPA generated was because of reaction condition on the enzyme MHETase W397A, and not on the ability of PETase to hydrolyze BHET. BHET was first incubated with PETase in at 30 °C for 2 hours, then the reaction was stopped by addition of 5 mM of PMSF and The PETase enzyme was removed from the reaction. The remaining solution after PETase removal was used to set up the MHET hydrolysis reaction by MHETase W397A. HPLC analyses of reaction products generated at different pH, and comparison of the amount of generated TPA to the TPA standard curve, revealed that MHETase W397A could hydrolyze MHET to TPA more efficiently at pH 7 than at pH 6 and pH 8 (Figure 7).
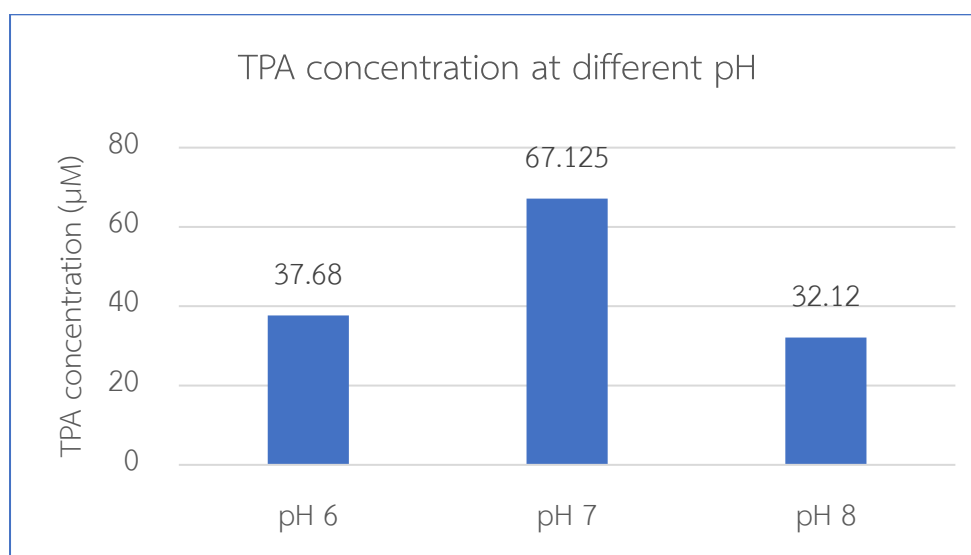

Figure 7: Concentrations of TPA generated from MHET hydrolysis by MHETase W397A at different pH. Highest concentration of TPA was observed at pH 7. TPA concentration was calculated by using TPA standard curve.

Discussion and Conclusion

The overall goal of this project is to characterize the MHETase enzyme variants and develop it for application in enzymatic plastic degradation. In this work, *Ideonella sakaiensis* MHETase W397A was recombinantly expressed in *E. coli* and purified using Ni-NTA chromatography. The purified MHETase W397A was shown to be active in the conversion of MHET to TPA, as judged by HPLC analysis.

To identify the optimal pH for MHET hydrolysis by MHETase W397A. The MHET hydrolysis reaction was set up at varying pH (pH 6-8). MHETase W397A could hydrolyze MHET to TPA more efficiently at pH 7 than at pH 6 and pH 8. Our finding showed a different from the previous study. They found that enzyme MHETase wildtype could hydrolyze MHET to TPA better at pH 8 than at pH 6 and 7

(Gottfried J. Palm, 2019). It might be due to some factors such as the buffer and the incubation temperature. In the previous study, they used 35 mM of phosphate buffer with 80 mM NaCl, and the incubation was at room temperature (Gottfried J. Palm, 2019). However, in our pH optimization experiment, we used 50 mM of phosphate buffer with 25 mM of Tris-HCl and incubated at 30 $^{\circ}$C.

In this study, we have successfully expressed and purified MHETase W397A variant and showed that the enzyme is active in MHET hydrolysis. The optimal pH for MHET hydrolysis by MHETase W397A is pH 7.0.

Next, we also plan to optimize the other reaction conditions, including temperature, and ionic strength. In addition, we will characterize the enzyme's tolerance to organic solvents and detergents.

For temperature optimization, the optimal pH 7 from the experiment will be used and the temperature will be monitor in the range between 10 to 70 °C. We choose these condition range in the MHETase W397A variant reaction buffer to study because the previous study showed good results in these condition range of wild-type MHETase (Gottfried J. Palm, 2019). For concentration of salt optimization, the optimal pH and temperature will be used, and the concentrations of sodium sulphate (Na_2SO_4) will be varied in the range between 0.3 to 2 M. We choose Na_2SO_4 to study in the reaction because it was found that Na_2SO_4 could increase the enzyme IsPETase activity, which has a similar function as MHETase in plastic degradation (Congcong Liu, 2018).

Once pH, temperature and ionic strength are optimized. Different kinds of organic solvents and detergents such as ethanol, propanol, Tween 20, Triton X-100, and SDS will be added to the reaction mixture to test the enzyme's stability. In enzyme's tolerance experiment, 5% to 10% of the different kinds of organic solvents will be added to the enzyme reaction to test the enzyme stability.

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References

Barth M, O. T. Effect of hydrolysis products on the enzymatic degradation of polyethylene terephthalate nanoparticles by a polyester hydrolase from Thermobifida fusca. Biochem Eng J. 2015; 93: 222-228.

Congcong Liu, e. a. Structural and functional characterization of polyethylene terephthalate hydrolase from Ideonella sakaiensis. Biochemical and Biophysical Research Communications. 2018

d'Ambri'eres, W. Plastics recycling worldwide: current overview and desirable changes. Field Actions Science Reports J. Field Actions. 2019; 12-21.

Garcial J.M., R. M. The future of plastics recycling. Science. 2017; 358: 870-872.

Gasteiger E., H. C. Protein Identification and Analysis Tools on the ExPASy Server. (In) John M.Walker (ed): The Proteomics Protocols Handbook, Humana Press. 2005; 571-607.

Gottfried J. Palm, L. R. Structure of the plastic-degrading Ideonella sakaiensis MHETase bound to a substrate. Nature Communications. 2019; 10.

Grigore, M. Methods of recycling, properities and applications of recycled thermoplastic polymers. Recycling. 2017; 2:24.

Leibly, D. J. Stabilizing Additives Added during Cell Lysis Aid in the Solubilization of Recombinant Proteins. PLoS ONE. 2012; 7: e52482.

Makoto Furukawa, e. a. Acceleration of Enzymatic Degradation of Poly (ethylene terephthalate) by Surface Coating with Anionic Surfactants. ChemSusChe. 2018; 11.

Müller, R. J. Enzymatic degradation of poly (ethylene terephthalate): rapid hydrolyse using a hydrolase from T. fusca. Macromolecular rapid communications. 2005; 26: 1400-1405.

Roser, H. R. (2018). Plastic Pollution. OurWorldInData.org., 'https://ourworldindata.org/plastic-pollution' [Online Resource]. Retrieved 2021, from https://ourworldindata.org/faq-on-plastics#how-much-plastic-and-waste-do-we-produce

Shosuke Yoshida, e. a. A bacterium that degrades and assimilates poly (ethylene terephthalate). Science. 2016; 1196-1199.