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Class I hydrophobins pretreatment stimulates PETase for monomers recycling of waste PETs

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ABSTRACT

Poly(ethylene terephthalate) hydrolase (PETase) from *Ideonella sakaiensis* 201-F6 was expressed and purified from *Escherichia coli* to hydrolyze poly(ethylene terephthalate) (PET) fibers waste for its monomers recycling. Hydrolysis carried out at pH 8 and 30 °C was found to be the optimal condition based on measured monomer mono(2-hydroxyethyl) terephthalate (MHET) and terephthalic acid (TPA) concentrations after 24 h reaction. The intermediate product bis(2-hydroxyethyl) terephthalate (BHET) was a good substrate for PETase because BHET released from PET hydrolysis was efficiently converted into MHET. Only a trace amount of MHET could be further hydrolyzed to TPA. Class I hydrophobins RolA from *Aspergillus oryzae* and HGFI from *Grifola frondosa* were expressed and purified from *E. coli* to pretreat PET surface for accelerating PETase hydrolysis against PET. The weight loss of hydrolyzed PET increased from approximately 18% to 34% after hydrophobins pretreatment. The releases of TPA and MHET from HGFI-pretreated PET were enhanced 48% and 62%, respectively. The selectivity (TPA/MHET ratio) of the hydrolysis reaction was approximately 0.5.

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1. Introduction

Global production of poly(ethylene terephthalate) (PET) was about 50 million metric tons annually [1] and it has been used in various industries such as packaging, textile, films, and containers [2–4]. PET recycling has become an important issue due to the public awareness of sustainable development of our society. As the most widespread synthetic fiber, PET fiber had a market share of around 51.5% from total global fiber production of 55.1 million metric tons in 2018 [5]. Most of the PET fibers produced are used by textile industry for the preparation of fabrics because of its desirable properties such as high strength, elasticity, durability, and extreme resistance to chemicals. Besides, PET fabrics also have benefits over natural fiber fabrics in less wrinkles, shrinking, abrasion, fast-drying, and lower cost [6,7].

Traditional methods for recycling waste PET are essentially downcycling processes. For example, PET drink bottles are re-melted to make lower grade containers or polyester fabrics because the recycling processes involve harsh chemical and physicochemical conditions usually induce degradation of its macromolecular structures that can negatively affect the properties of the recycled PET. Transforming PET back into its monomers terephthalic acid (TPA) and ethylene glycol (EG) for repolymerization is an attractive alternative PET upcycling method. Several chemical methods for PET upcycling such as glycolysis, methanolysis, and aminolysis have been developed [8]. However, these chemical methods generally involve energy-intensive processes and generate additional pollutants. In recent times, enzymatic monomer recycling of PET has gained great attention in industries due to its eco-friendly and mild reaction conditions [7,9,10]. Various hydrolases, such as esterase, lipase, cutinase, and poly(ethylene terephthalate) hydrolase (PETase) have demonstrated PET hydrolysis activity at different extents [11–13]. Among these enzymes, only cutinase and PETase are considered to have potential industrial applications due to their relatively higher PET hydrolysis activity.

Cutinase, discovered more than 40 years ago from fungi responsible for plant pathologies attacks and hydrolyzes cutin which is a complex hydrophobic waxy polyester covers aerial surface of plants. Only recently, cutinases from various strains were found to be potential PET hydrolysis enzymes [11]. It has been reported that the access of active site of cutinase and binding of cutinase to the insoluble PET are the main reaction rate-limiting factors [7,14] because mutations on cutinase to enlarge the active site area was demonstrated can increase its PET hydrolysis rate. Several genetic modification studies on cutinase structure have shown improved catalytic activities and thermostability [15–18]. In addition, employing additives such as synthetic surfactant [19] and natural surfactant hydrophobin [20] in cutinase hydrolysis systems to accelerate PET hydrolysis kinetic have been reported. These additives could transform the hydrophobic surface of PET substrate into hydrophilic which facilitates the contact and binding of cutinase to the PET surface.

In contrast, PETase is a quite newly discovered enzyme by Yoshida et al. [21] in 2016 from gram-negative *Ideonella sakaiensis* 201-F6,

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with unusual ability to utilize PET as substrate for growth [15,16,22]. PETase is a high PET-specificity hydrolase can effectively degrade PET at moderate conditions and has also evolved to degrade crystalline PET. It exhibits more open active-site cleft than that of cutinases leads to its capability on degrading other semiaromatic polyesters but not aliphatic polyesters [23]. Several PETase variants prepared by site-direct mutagenesis of its active sites have shown to improve its PET-degrading activity [24].

High crystallinity and hydrophobicity of PET were reported to limit its enzymatic degradation [3,17,18,25]. Since PETase is capable to hydrolyze crystalline PET, in this study, we purified wild type PETase of *Ideonella sakaiensis* expressed in *Escherichia coli* to hydrolyze semi-crystalline PET fiber and high-crystalline PET bottle for the monomers recycling of PET. Cationic surfactant and amphiphilic hydrophobins pretreatment on PET surfaces have shown to be degraded by cutinase at a much faster rate. The enhanced degradation rates were explained based on the increased enzymes affinity toward PET surface resulted from the interactions with surface bonded cationic surfactant and hydrophobins [19,20]. Hydrophobins are amphiphilic proteins consist of eight cysteine residues and can self-assemble on hydrophilic-hydrophobic interfaces then reversing the substrate surface properties [26–28]. These surface-active fungal proteins are classified into class I and class II according to their hydropathy patterns [29,30]. Class I hydrophobins have characteristics of rodlet structure and insoluble formation which can only be dissociated in strong acid, while the film formed by class II hydrophobins are soluble in mild conditions such as ethanol or sodium dodecyl sulfate (SDS) [31,32]. The effect of class I hydrophobin on accelerating PET fibers waste degradation by PETase has never been reported yet. In this work, class I hydrophobins RolA from *Aspergillus oryzae* and HGFI from *Grifola frondosa* were expressed in *E. coli* and purified for pretreating PET to study their effect on accelerating PETase hydrolysis for recycling PET monomers terephthalic acid (TPA) and mono(2-hydroxyethyl) terephthalate (MHET).

2. Materials and methods

2.1. Materials and chemicals

PET fiber was kindly provided by Lealea Enterprise (Taipei, Taiwan) and high-crystallinity PET was obtained from drinking water bottle. Surface area of the PET fibers and PET bottle powder were analyzed using surface area and pore size analyzer (BEL Japan, Inc.). The crystallinity of PET substrates was analyzed using X-Ray Diffraction (XRD) (D2 phaser, Bruker, Germany). Acetonitrile of HPLC grade was obtained from Merck. *p*-Nitrophenol (pNP) and *p*-nitrophenyl acetate (pNPA) were obtained from Sigma-Aldrich (USA) and Alfa Aesar (Massachusetts, USA), respectively. All other reagents used were analytical grade available from Acros, Merck, and Sigma-Aldrich.

2.2. Plasmids, strains, and media

Plasmid pET-21b(+) carrying PETase from *Ideonella sakaiensis* 201-F6 (Genbank GAP38373.1) was transformed into host *E. coli* SoluBL21 for the expression as described in our previous study [33]. Class I hydrophobins RolA from *Aspergillus oryzae* (Genbank XM_001824829.3) and HGFI from *Grifola frondosa* (Genbank EF486307.1) were constructed into plasmids pET-24a-rola and pET-24a-hgfI, respectively and expressed in *E. coli* SoluBL21. *E. coli* DH5α and SoluBL21 competent cells (Yeastern Biotech, Taiwan) were used for cloning and expression, respectively.

2.3. Expression and purification of proteins

A single colony of each recombinant *E. coli* clone was grown in 3 mL of Luria Bertani (LB) broth consisted of 50 mg/mL kanamycin for the expression of hydrophobins and 50 mg/mL ampicillin for the PETase, incubated overnight at 37 °C with shaking at 200 rpm. Each preculture was poured into 50 mL of fresh LB and incubated at 37 °C until the absorbance of the cultures (A_{600}) reached ~0.7. The protein expression was induced by adding 0.5 mM Isopropyl-beta-D-thiogalactopyranoside (IPTG) at 22 °C for 16 h. Bacterial pellets were recovered using centrifugation at 6000 ×*g* 4 °C for 15 min, washed twice with double-distilled water (ddH_2O), and dispersed in lysis buffer. *E. coli* cells pellet was disrupted by ultrasonicator for 15 min (10 s on pulse and 20 s off pulse). After centrifugation, the soluble fraction of PETase was purified by immobilized metal-chelated affinity chromatography (IMAC) under non-denaturing conditions as described in our previous work [33]. While the insoluble fractions of the crude extract of expressed hydrophobins were dissolved with 1.5 mL of denaturing solution containing guanidine-HCl 6 M and kept at 4 °C overnight. The solubilized proteins were purified by IMAC under denaturing conditions and the eluted proteins were washed with PBS buffer using ultrafiltration spin column (10 kDa) for 5 times to remove denaturant as shown in the purification scheme (Fig. S1). SDS-PAGE analysis was used to estimate the molecular weight of all sample fractions. The concentration of purified proteins was measured using Bradford protein assay.

2.4. PETase activity assay

pNPA was used to analyze esterase activity of the purified recombinant PETase. The hydrolysis of pNPA releases pNP, which can be measured spectrophotometrically at 410 nm. PETase concentration of 1.5 μM and pNPA concentrations of 0.05 to 1.5 mM were used to determine the reaction kinetic parameters at 30 °C and pH 8.

2.5. Surface tension and water contact angle (WCA) measurement

Surface tensions of hydrophobin solutions were measured using interfacial tensiometer (OCA 15EC, Japan) based on pendant drop method. The images of performed liquid drops of hydrophobins (50 μg/mL) dissolved in ddH_2O were recorded. WCA analysis was performed on hydrophobic PET fiber and PET bottle powder surfaces by measuring contact angle using Goniometer Model 100SB (Sindatek Instruments Co., Ltd, Taiwan). Each substrate was coated with 50 μL of 0.5 μM hydrophobins and incubated at 30 °C overnight. Then the surfaces were rinsed twice with distilled water and dried under a stream of nitrogen. The measurements were carried out in triplicate on different locations on the surface of substrates.

2.6. Enzymatic hydrolysis of PET

Before enzymatic hydrolysis, all PET substrates were cleaned by rinsing with 1% sodium dodecyl sulfate (SDS), followed by ethanol (70 v/v%) and distilled water, then dried at 50 °C for 1 day. To study the effect of hydrophobin on PET hydrolysis, the cleaned PET substrates of 3 mg were mixed in 200 μL phosphate buffer containing 20 μM of hydrophobins at 30 °C for 3 h. PETase enzyme solutions prepared in 50 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4) were then loaded for PET hydrolysis. The effect of hydrolysis pH (phosphate buffer 6.5–9.5), temperature (20–50 °C), and PETase enzyme loading amount (1–25 ppm) on PET hydrolysis were carried out in orbital shaker at 200 rpm for 24 h. Then, the hydrolyzed products in the supernatants were analyzed using high-performance liquid chromatography (HPLC). Weight loss of PET substrates after 5 days hydrolysis was determined by measuring the weight of solid residue collected by centrifugation and washed with copious distilled water and dried at 50 °C for 1 day. The morphology of untreated and treated PET fiber samples was analyzed by field emission scanning electron microscope (SEM) (JSM-6500F, JEOL, Japan).

2.7. HPLC analysis

PET hydrolysis products were separated using a Shimadzu 10A HPLC System equipped with UV–Vis detector (SPD-10A) and ODS hypersil C18 column (Thermo Scientific™). An isocratic mobile phase consisting of 70% water, 20% acetonitrile, and 10% formic acid (v/v) was used at a flow rate of 1 mL/min. The concentrations of hydrolyzed products (BHET, MHET, and TPA) were detected at 254 nm and calculated from the areas of the adsorption peaks using calibration curves established from TPA and BHET standard solutions. The retention time of TPA, MHET, and BHET was about 4.4, 5.0, and 5.9 min, respectively (Fig. S2). The MHET standard solutions were prepared by hydrolyzing BHET standard solution using PETase. As shown in Fig. S3, at the end of reaction most of BHET was converted into MHET, only appreciable amount of TPA was produced. Based on the amount of BHET consumed and TPA generated, MHET concentration in the standard solution was determined.

2.8. Statistical analysis

All the samples for hydrolysis were performed in triplicate. Sigma Plot 12.5 (Systat Software Inc., USA) was used to determine error bars using standard deviation method.

3. Results and discussion

3.1. Expression and purification of recombinant proteins

As reported in our previous work [33], PETase was expressed as a soluble protein after 16 h IPTG induction at 22 °C cultivation. After IMAC purification, an apparent band with a molecular weight of ~30 kDa was observed. This band was considered as purified PETase and demonstrated high activity against pNPA. The purified PETase showed K_m of 36 μmol^{-1} L and k_{cat} of 1.01 s^{-1} using pNPA as substrate.

Hydrophobin RolA from *Aspergillus oryzae* and HGFI from *Grifola frondosa* were successfully expressed in *E. coli* soluBL21. As shown in Fig. S4, HGFI was expressed after 16 h IPTG induction at 22 °C as an insoluble protein with a molecular weight of ~14 kDa. Both RolA and HGFI belong to class I hydrophobins that were known to have very unique characteristics such as the formation of insoluble form, amyloid fibrils, and fibrillar rodlets [34]. As a consequence, the insoluble expressed RolA and HGFI in *E. coli* were not unexpected. The process for purification of insoluble hydrophobins is shown in Fig. S1. The insoluble fraction of disrupted cells suspension was solubilized in 6 M guanidine hydrochloride overnight. After clarified by centrifugation, the supernatant was loaded into an IMAC resin column for hydrophobins purification because a 6xHis tag was constructed to fuse at C-terminal of their sequence. As shown in Fig. S5, an apparent band of RolA and HGFI could be observed at ~11 and ~14 kDa, respectively that corresponding well with their putative size based on their gene sequences. Approximately, 1.5 mg RolA and 2.2 mg HGFI were purified from 50 mL of recombinant *E. coli* cultures.

3.2. Properties of expressed hydrophobins

Since one main characteristic of hydrophobins is its surface-active ability to reduce surface tension of aqueous solution, the effect of expressed hydrophobins on reducing water surface tension was checked to ensure the recovery of their function from denatured insoluble form. Pure water has a surface tension of 70 mN/m, as shown in Fig. 1, hydrophobin RolA and HGFI could significantly reduce surface tension of water to 28.75 mN/m and 24.89 mN/m, respectively which is quite close to what obtained in our previous work with native hydrophobin RolA (31.78 mN/m) [33]. Evidently, the surface-active function of the expressed hydrophobins could be mostly recovered from IMAC purification of the solubilized hydrophobins.

The surface-active function of the expressed hydrophobins on wetting the surfaces of PET fiber and PET powder from drinking bottle was also studied. Water contact angle of RolA and HGFI treated substrate surfaces was measured. As shown in Fig. 2, WCA of pristine PET fiber was 95.22 ± 2°, while PET powder was 107.89 ± 6°. Apparently, both of these two PET substrates possess a hydrophobic surface. After RolA and HGFI pretreatment, the contact angle on PET fiber surface significantly decreased to 24.79 ± 3° and 18.68 ± 3°, respectively. The contact angle on PET powder surface decreased to 30.61 ± 4° and 23.22 ± 5°, respectively. The significant contact angle decrease after hydrophobin pretreatment indicates that both hydrophobins can effectively transform the hydrophobic surface of PET into hydrophilic due to the amphiphilic property of hydrophobins. In other words, hydrophobin will self-assemble on PET surface to have its hydrophobic portion intimately contact with pristine PET surface and expose its hydrophilic portion [35].

3.3. PET hydrolysis by PETase

The activity of IMAC purified PETase for hydrolyzing PET to release water-soluble monomers was investigated using PET fiber and PET bottle powder as substrates. By hydrolyzing PET with PETase, MHET, TPA, and BHET will be produced as shown in Fig. 3. HPLC analysis of the hydrolysis product shows that baseline separation could be achieved for these monomers with TPA appeared first followed by MHET and BHET (Fig. S2). MHET standard was obtained by hydrolyzing BHET with PETase. As shown in Fig. S6, MHET peak was generated from BHET

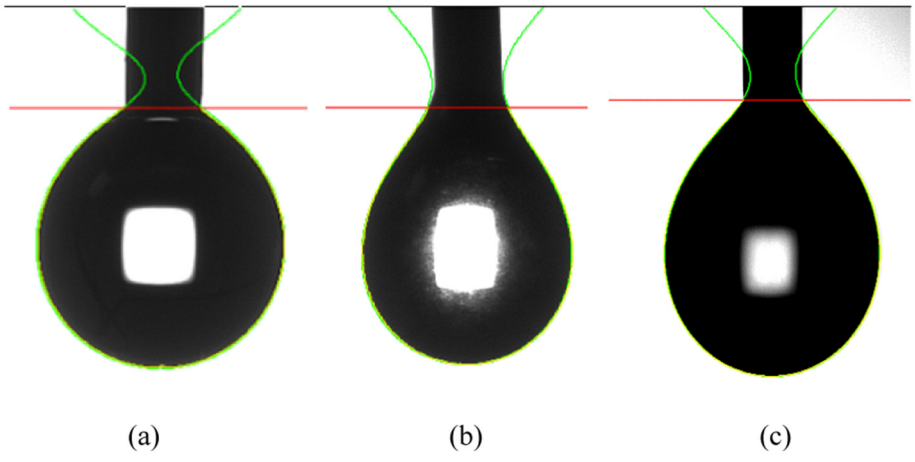

Fig. 1. Surface tension measurements using pendant drop method. (a) Pendant droplet of pure water, (b) 50 μM HGFI, (c) 50 μM RolA in water.

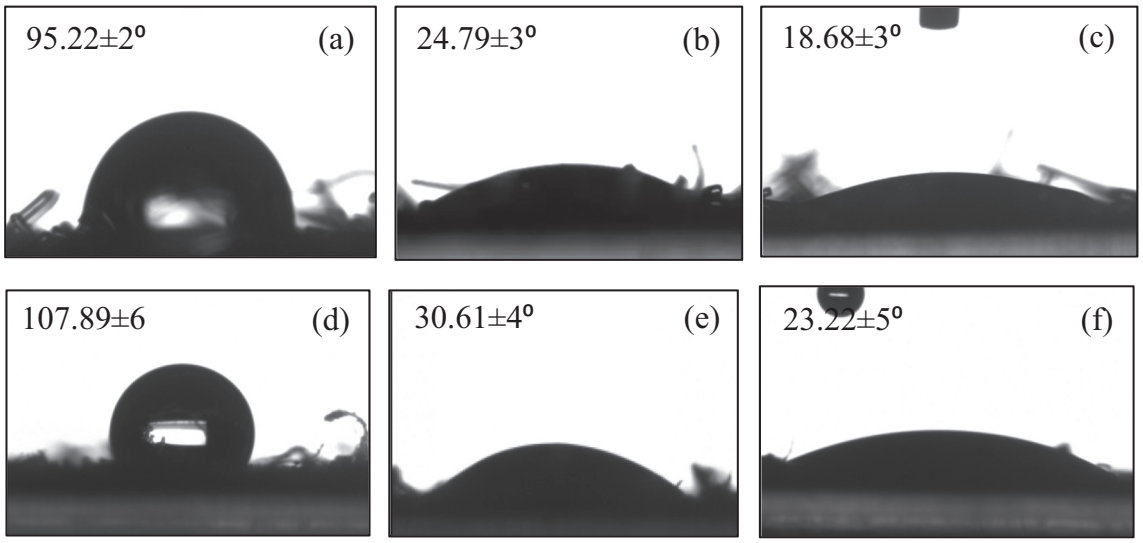

Fig. 2. Water contact angle (WCA) of (a) untreated PET fiber, (b) RolA-treated PET fiber, (c) HGFI-treated PET fiber, (d) untreated PET powder, (e) RolA-treated PET powder, (f) HGFI-treated PET powder.

after 30 min reaction with PETase. A small but apparent TPA peak was observed after 1 h. After 7 h reaction, BHET was nearly consumed and only appreciable TPA increase was observed. Evidently, PETase can effectively hydrolyze BHET into MHET but further hydrolyzing MHET into TPA is very inefficient.

PET fiber was first hydrolyzed by 5 ppm PETase at pH 8 for 24 h with temperature ranging from 20 to 50 °C. As shown in Fig. 4A, a significant amount of TPA and MHET were released during the reaction. In contrast, only a trace amount of BHET could be detected. BHET should be ever produced in the PET hydrolysis process based on mechanism reported by Joo et al. [36] but it was further hydrolyzed into MHET as shown in Fig. S6. Also demonstrated in Fig. S6, MHET still can be hydrolyzed by PETase into TPA but at very slow rate, the significant amount of TPA observed was mostly resulted from terminal digestion of the TPA-terminal PET fragments, one of two fragments generated at the nick generation step as proposed by Joo et al. [36]. As a consequence, only MHET, TPA, and EG will be accumulated in PETase degradation process after 24 h. TPA and MHET concentrations of 1.56 ± 0.01 mM and 4.03 ± 0.06 mM were obtained at 30 °C, respectively. At temperature over 35 °C, a gradual decrease in concentration occurred. This indicates that the PETase started to deactivate at temperature higher than 30 °C because PETase is originated from a mesophilic bacterium, *Ideonella sakaiensis*. Its stability cannot be extended to a higher temperature [24,37]. Therefore, 30 °C was selected for further study on PET hydrolysis using PETase.

Phosphate buffer of 50 mM with different pH (6.5–9.5) was employed for PET hydrolysis using 5 ppm PETase at 30 °C for 24 h. As shown in Fig. 4B, TPA and MHET concentration reaches highest level of 1.66 ± 0.03 mM and 4.13 ± 0.01 mM at pH 8, only appreciable BHET could be detected. Liu et al. also reported the highest activity PETase was obtained at pH 8 [38]. MHET concentration is approximately 2.5 fold higher than that of TPA (TPA/MHET ratio of 0.4) after 24 h. The higher MHET fraction in the monomers product is because MHET can be released not only from the HE-terminal PET fragments generated from initial nicking step but also from the other hydrolysis product BHET,

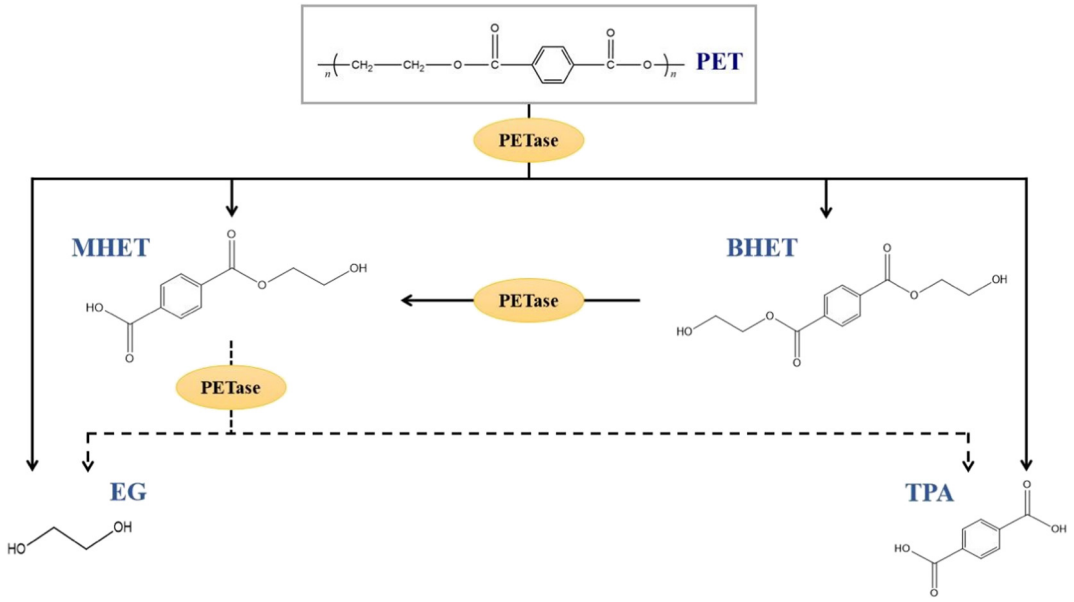

Fig. 3. PETase catalyzed PET hydrolysis products (solid line: major reaction, dotted line: minor reaction).

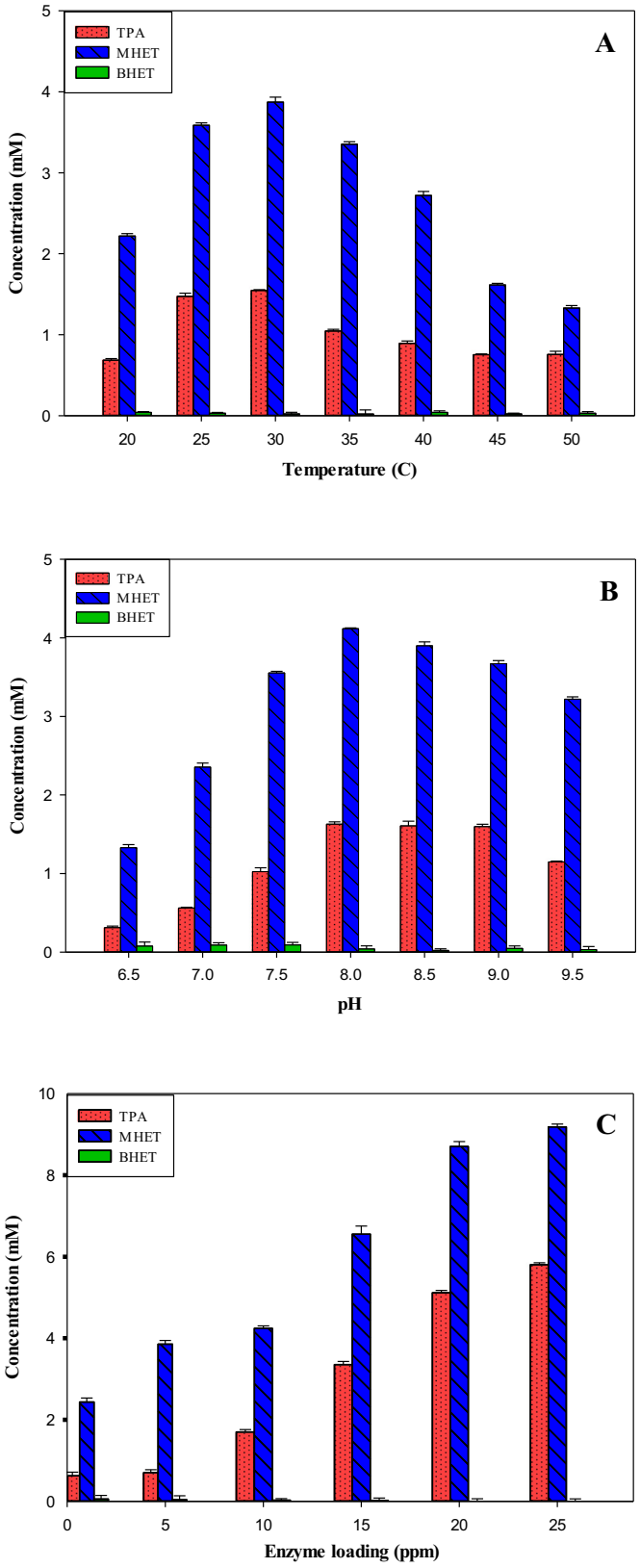

Fig. 4. The effect of (A) temperature, (B) pH, (C) PETase loading amount on PET fiber hydrolysis by PETase for 24 h.

$(MHET)_2$, and 2-HE$(MHET)_2$. On the other hand, TPA can only release from TPA-terminal PET fragments and $(MHET)_2$ according to the Joo's model [36].

It is interesting to be noted that ratio of TPA to MHET increased with the amount of PETase loaded for PET degradation. As shown in Fig. 4C, 5.18 ± 0.06 mM TPA and 8.82 ± 0.12 mM MHET (TPA/MHET ratio of 0.6) were obtained at 20 ppm of PETase. It is not clear why TPA fraction will increase with PETase loading amount. Based on Joo's model [36], the number of nicks generated in the PET degradation process will increase with the amount of PETase loaded. With increased amount of TPA- and HE-terminal PET fragments, probably, PETase activity for releasing terminal TPA is more effectively enhanced as compared with its activity for generating MHET related intermediates from HE-terminal fragments. In other words, TPA releasing activity of PETase is more concentration dependent than its MHET releasing activity.

3.4. Effect of hydrophobin on PET hydrolysis

PETase is reported to have a higher PET degradation rate as compared with other PET degradation enzymes, probably due to its hydrophobic affinity toward PET via the flat hydrophobic surface found in its substrate binding cleft [36]. The feasibility of using hydrophobins pretreatment to enhance PET degradation by PETase was studied. PET fiber with and without hydrophobin pretreatment were incubated with PETase at 30 °C using protein loading of 20 ppm and pH 8. As shown in Fig. 5, TPA and MHET concentrations were the main monomer

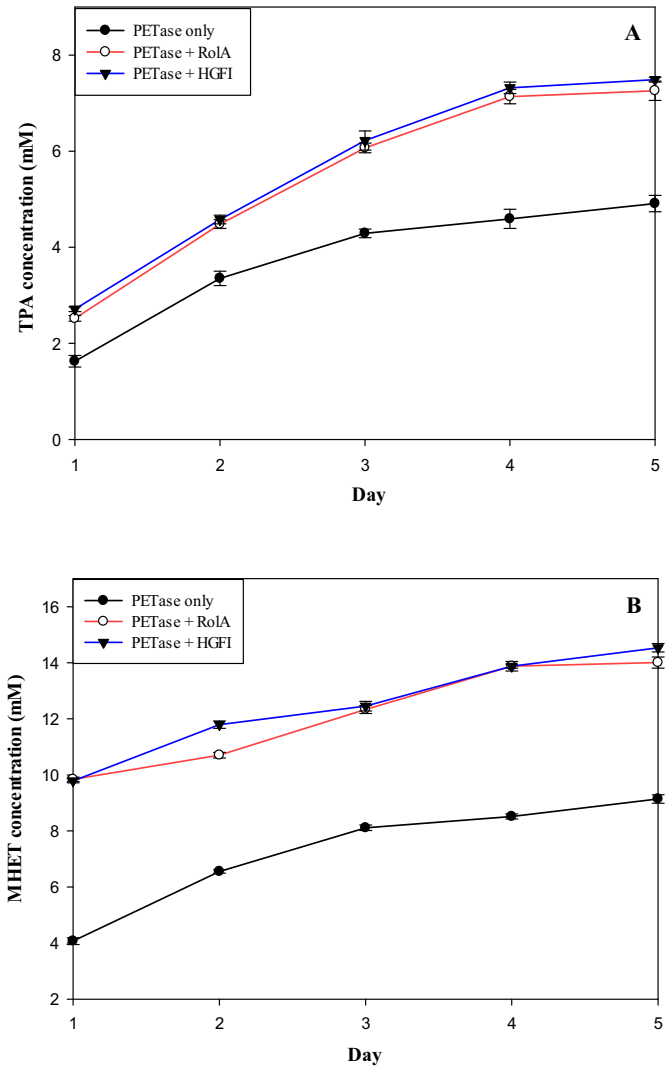

Fig. 5. Concentration of (A) TPA and (B) MHET released upon PET fiber hydrolysis by PETase and hydrophobins-treated PET. The enzymatic reactions were performed at 30 °C pH 8 and enzyme loading of 20 ppm.

products and increased near-linearly with time during first 4 days. Both monomers concentrations significantly increased when PET substrates were pretreated either with hydrophobins RolA or HGFI. These two hydrophobins pretreatment only resulted in appreciable difference in stimulating monomers releasing from PET hydrolysis. After 5 days reaction, TPA concentration achieved a 48% increase from 5.17 ± 0.17 mM to 7.60 ± 0.2 mM, whereas MHET reached a 62% enhancement from 9.12 ± 0.12 mM to 14.65 ± 0.15 mM. MHET release seems to be more effectively stimulated by hydrophobin HGFI pretreatment that leads to the reduction of TPA to MHET ratio from 0.56 to 0.51 (Table S2). In other words, the presence of hydrophobin on surface may interfere the digestion of TPA-terminal fragments for TPA releasing and resulted in a reduced TPA to MHET ratio.

Simultaneous addition of hydrophobins with PETase, however, did not yield significant enhancement (data not shown). This indicates that hydrophobins need a longer time to well assemble on PET surface. Without well-assembled hydrophobins on the surface of PET, the affinity binding of PETase to PET would not be enhanced to stimulate the hydrolysis process. Previous results of PET degradation by cutinase with class II hydrophobin have been reported by Espino-Rammer et al. [39]. In their work, hydrophobin was added simultaneously with cutinase and hydrophobin was considered as a surfactant that interacted with cutinase so that its conformation will be changed that leaded to a maximal 2.5 fold higher extent of stimulation. The binding of surfactant to cutinase [40] and stimulation effects of surfactant on cutinase PET hydrolysis [41] have also been reported, respectively. In this work, we did not observe stimulation effect when hydrophobins were added simultaneously with PETase for hydrolysis. Approximately, a stimulation extent of 1.5 fold was observed for the hydrophobins treated PET hydrolysis by PETase. Evidently, the stimulation mechanism of class I hydrophobins with PETase is different from class II hydrophobins with cutinase on PET hydrolysis. The stimulation effect of class I hydrophobins was interpreted based on the discussion described by Takahashi et al. [42] that a reduction of the surface tensions between PET substrate and PETase due to the self-assembled and bounded hydrophobins. PETase would then accumulate at the interface between PET surface and the water phase and thus exhibit increased activities on the substrates. In other words, PETase concentration will be enhanced at the more hydrophilic hydrophobin modified PET surface.

As shown in Fig. 6A, the semi-crystalline PET fiber was significantly degraded (12.4%) within 1 day, then the degradation rate gradually decreased and reached a maximum weight loss of 18.4%. In contrast, degradation of PET fiber was enhanced by hydrophobin RolA and HGFI pretreatment and maximum weight loss of 31.4% and 34.6% were achieved after 5 days. As a comparison, high-crystalline PET powder from drinking bottle was also degraded with PETase under the same conditions as for PET fiber. As shown in Fig. 6B, the weight loss of PET powder significantly increased in 1st day then the weight loss rate gradually decreased and reached the maximum value of 17.3% which is similar to our previously reported [33]. A maximum weight loss of 27.6% could be achieved after pretreatment with hydrophobin RolA, while the HGFI showed a maximum weight loss of 29.2%. PET fiber showed the higher degradation by PETase is probably due to its lower crystallinity. Crystallinity is one of important factors affecting the enzymatic hydrolysis, lower crystallinity increases the movement of polymer chains and increases the accessibility of enzyme on substrates [3]. As shown in Fig. S7 and Table S1, PET fiber has a lower crystallinity (38.8%) as compared to PET bottle powder (64.8%). Evidently, the lower crystallinity facilitated the hydrolysis of PET by PETase. Although the surface area of PET powder was higher than PET fiber, the surface area has a minor effect on hydrolysis compared to the crystallinity of PET. SEM images of HGFI-pretreated PET fiber samples before and after PETase enzyme attacked are shown in Fig. 7. Evidently, PETase enzyme caused apparent roughening and cracks on the semi-crystalline PET surface after 5 days degradation. XRD of the samples shows that the crystallinity of PETase degraded PET fiber increased that indicates the crystalline fraction of PET were not susceptible to enzymatic attacks.

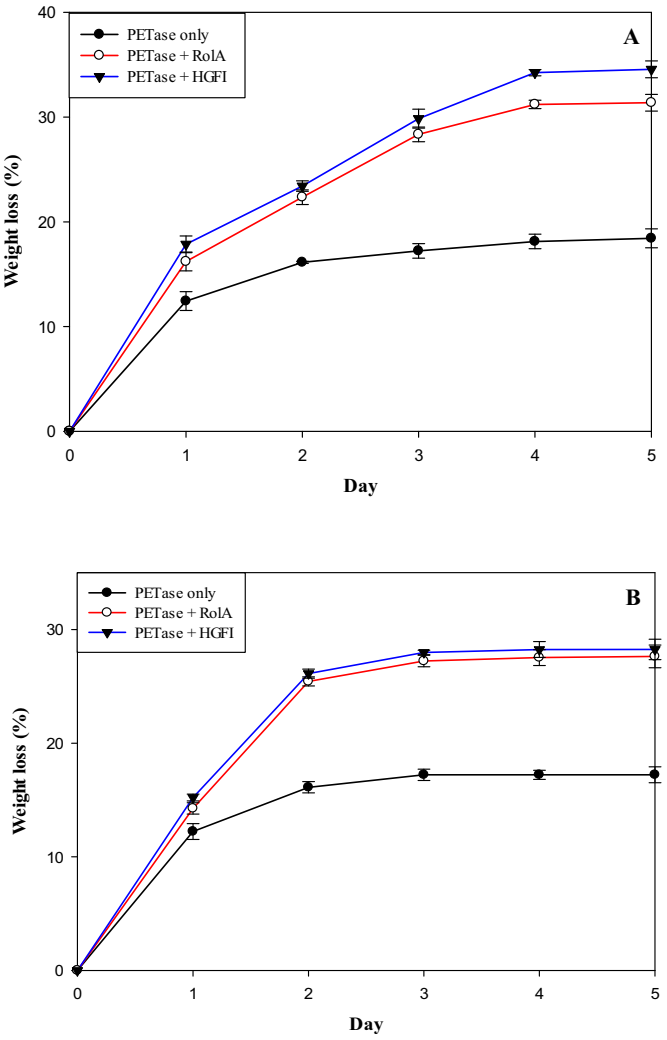

Fig. 6. Weight loss of hydropobins treated (A) PET fiber (B) PET powder during 5 days of hydrolysis by PETase.

4. Conclusions

In this work, we have successfully expressed and purified class I hydrophobins RolA (~11 kDa) and HGFI (~14 kDa) in *Escherichia coli*. The surface-active proteins could enhance PETase hydrolysis of semi-crystalline PET fiber and high-crystalline PET bottle. We investigated the optimal conditions of hydrolysis reaction at 30 °C, pH 8 using PETase enzyme loading of 20 ppm showed the highest biodegradability performance on PET fiber due to its preference to attack the substrates at amorphous regions. Moreover, the surface modification of PET fiber with recombinant HGFI achieved the highest TPA and MHET products and the weight loss of up to 34.56% after 5 days hydrolysis. Our findings suggest that the interaction of self-assembled class I hydrophobins and hydrophobic PET is an important step toward enhancing the hydrolysis rate of PET fiber recycling.

CRediT authorship contribution statement

Nathania Puspitasari: Methodology, Investigation, Software, Validation, Visualization, Data Curation, Writing - Original draft

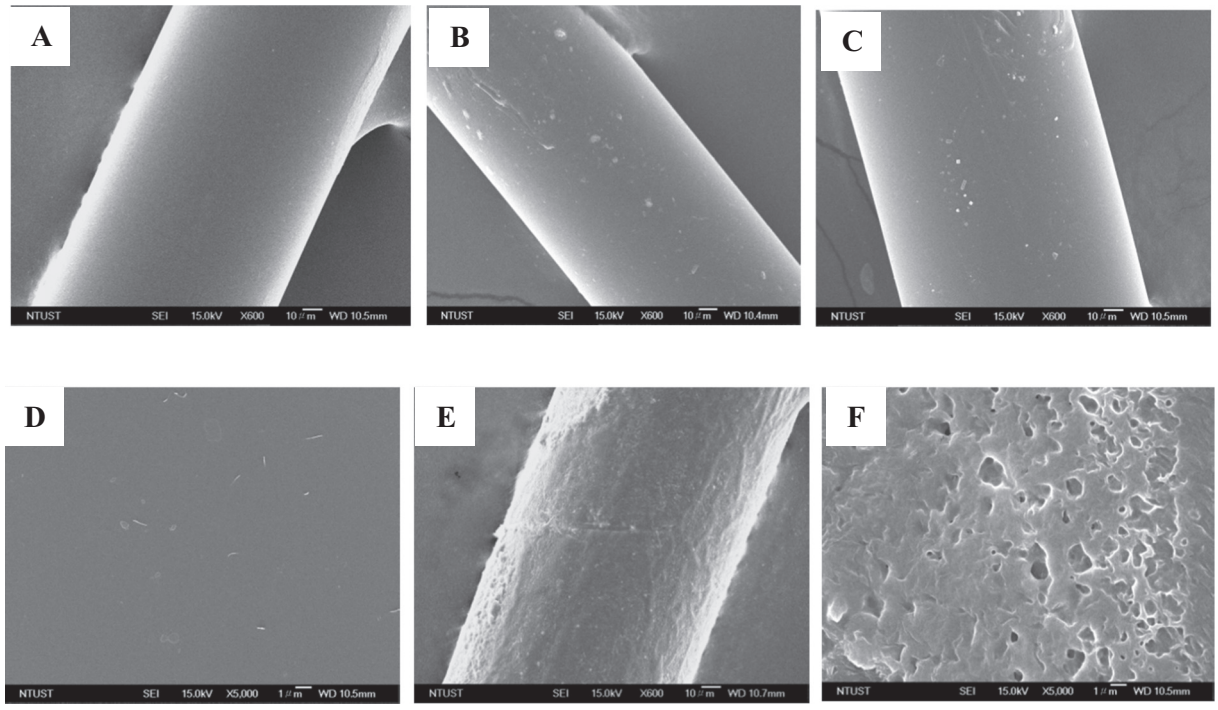

Fig. 7. SEM images of PET fiber surface after 5 days PETase hydrolysis (A) untreated PET fiber, (B) HGFI-pretreated PET fiber at 600×, (C) RolA-pretreated PET fiber at 600×, (D) HGFI-pretreated PET fiber at 5000×, (E) HGFI-treated followed by PETase hydrolysis at 600×, and (F) HGFI-treated followed by PETase hydrolysis at 5000× magnifications.

Shen-Long Tsai: Conceptualization, Writing - Reviewing & editing, Supervision
Cheng-Kang Lee: Conceptualization, Methodology, Writing - Reviewing & editing, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.02.026.

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