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Characterization of a cutinase from *Myceliophthora thermophila* and its application in polyester hydrolysis and deinking process

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ABSTRACT

A gene encoding cutinase (MtCUT) from *Myceliophthora thermophila* was expressed in *Pichia pastoris*. The optimal temperature and pH for MtCUT were 30 °C and 8.5, respectively. MtCUT showed high activities toward a broad range of *p*-nitrophenyl esters, and the highest specific activity was recorded for *p*-nitrophenyl butyrate. MtCUT hydrolyzed and broke down apple cutin into C16 and C18 family fatty acids. MtCUT could efficiently degrade polycaprolactone (PCL), the weight loss of which was 78.5% in 36 h. In addition, MtCUT could degrade PVAc, a kind of adhesive that is frequently used in papermaking and in synthetic toner or ink. Its performance in the deinking of waste papers was investigated and compared with that of commercial lipase in this study. The results showed that an ink removal efficiency of 78.4% on laser-printed paper and 81.3% on newspaper was obtained using MtCUT at 30 °C, which was better than that of lipase from *Candida rugosa* (CrLIP) (77.0% and 76.0%, respectively). The properties of the MtCUT-treated samples were similar to that of the control samples. This revealed that the cutinase MtCUT from *M. thermophila* has a great potential in PCL hydrolysis and the deinking process.

1. Introduction

Cutinases (3.1.1.74) are the smallest members of the α/β hydrolase family, which can hydrolyze the ester bonds of cutin, the insoluble biopolyester matrix in plant surfaces [1]. Cutinases were identified and characterized mainly from fungi and several bacteria [2,3]. Among them, the cutinase from *Fusarium solani* pisi was the most studied and widely used in industrial application [4]. The biochemical properties of cutinases from other fungi such as *Alternaria brassicicola* [5], *Aspergillus niger* [6], *Fusarium oxysporum* [7], *Monilinia fructicola* [8], *Sirococcus conigenus* [9], *Trichoderma reesei* [10], and *Thielavia terrestris* [11] have been studied in recent years, and it is believed that the cutinase-catalyzed hydrolysis of the cuticular layer is essential for fungal initial attack on plants [12].

Structural analyses of cutinase crystals show that their catalytic triad is composed of Ser-His-Asp, in which the catalytic serine is exposed to solvent [7,10,13,14]. Contrary to lipases, cutinases do not display or display little interfacial activation. Cutinases can act on water-soluble esters such as *p*-nitrophenyl esters and insoluble triglycerides, with a preference for short-chain substrates.

Cutinases have received much attention because of their potential applications in the degradation of aliphatic and aromatic polyesters [3]. Cutinase 1 from *Thermobifida cellulosilytica* was reported to hydrolyze several polyethylene terephthalate (PET) substrates [15] and the environmentally friendly polyester polyethylene furanoate (PEF) [16]. The cutinase from *Thielavia terrestris* was able to successfully hydrolyze polycaprolactone (PCL) and polybutylene succinate (PBS) [17]. Cutinases from other fungi, such as *F. solani*, *A. oryzae*, *A. brassicicola*, and *Humicola insolens*, were also reported to hydrolyze PBS [18]. The latter was also demonstrated to be active in the hydrolysis of polylactic acid (PLA) [19] or deacetylation of polyvinyl acetate (PVAc) [20].

Waste papers are now the major fiber resources for paper manufactures in the world. In the waste paper recycling process, the printed ink in the paper needs to be removed by a deinking process. Previous reports had demonstrated the successful use of various enzymes for waste paper deinking, such as cellulases, xylanases, pectinases, amylases, lipase, and laccases [21]. Although enzymatic treatment has shown some advantages over chemical deinking, further efforts are required to discover more effective enzymes for commercial applications [22]. Various polymers, such as polyvinyl acetate and polyacrylate with ester bonds, have been widely used as adhesives in papermaking or as binders in synthetic toner or ink [23]. The deinking ability of the lipases is due to the hydrolysis of triglycerides in vegetable oil-based inks into di- and monoglycerides and glycerol, thereby

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dislodging the ink particles from waste paper [24]. Thus, we speculate that cutinases could be used effectively in the deinking process because of their multiple capacities in the degradation of polyester binders, water-insoluble triglycerides, and soluble esters.

In this paper, a cutinase from *Myceliophthora* (*Thermothelomyces*) *thermophila* ATCC 42464 was heterologously expressed in *Pichia pastoris* (*Komagataella phaffii*), and its recombinant protein was efficiently purified in a single step using Ni-NTA affinity chromatography. The properties of this enzyme and its potential in the deinking of laser-printed paper and newspaper were investigated. To the best of our knowledge, it is the first report on the characterization and deinking application of cutinase from the species *M. thermophila*.

2. Materials and methods

2.1. Strains, vectors, and chemicals

Escherichia coli strain DH5α was used for the recombinant plasmid construction and propagation. The pPICZαA vector was used for cloning in *E. coli* and expression in *P. pastoris* strain KM71H. Commercial lipase (CrLIP) from *Candida rugosa* was purchased from Sigma Chemical Co. (St. Louis, MO, USA). *p*NP esters including *p*-nitrophenyl propionate (*p*NPP), *p*-nitrophenyl butyrate (*p*NPB), *p*-nitrophenyl valerate (*p*NPV), *p*-nitrophenyl octanoate (*p*NPO), *p*-nitrophenyl myristate (*p*NPM), and *p*-nitrophenyl stearate (*p*NPS) were products of Sigma. PBS was obtained from Anqing HeXing Chemical Corp. Ltd. (Anhui, China). PCL with a viscosity-average molecular weight of 80000 was supplied by Suzhou Zhong Zhicheng Plasticizing Co., Ltd (Suzhou, Jiangsu, China). PLA resin (4032D) was purchased from Nature Works Company (Minnetonka, MN, USA). Poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P-(3, 4) HB) containing 5% 4-HB content was purchased from Tianjin Green Bio-science Co., Ltd. (Tianjin, China). PVAc was provided by Jiangsu Yinyang Gumbase Materials, Co., Ltd. (Suzhou, Jintan, China). Other chemicals and reagents used in the study were of analytical grade and commercially available. All culture media were prepared according to the EasySelect™ *Pichia* expression system manual (Invitrogen, Carlsbad, CA, USA).

2.2. Expression and purification of MtCUT

The gene encoding *M. thermophila* ATCC 42464 cutinase (MtCUT) (accession no. XP_003663956.1) was synthesized by Springen Biotech Co. (Jiangsu, China), using optimized codons of *P. pastoris* (accession no. MF537432). This gene was ligated into the plasmid pPICZαA at the *Eco*R I/*Xba* I sites. To facilitate purification, a 6-histidine tag was fused at the C-terminus. The recombinant plasmid pPICZαA-MtCUT was transformed into KM71H strain by electroporation using a Genepulser II apparatus (Bio-Rad, Hercules, USA). The recombinant protein was induced by adding methanol to a final concentration of 1.0% every 24 h for a further 4 days and purified using Ni-NTA Agarose gel (Qiagen, Valencia, CA, USA). The homogeneity and molecular mass were estimated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [12% (w/v)].

2.3. Enzyme and protein assays

Cutinase activity was determined as below. Briefly, the appropriately diluted enzyme (0.075 μg of protein) was added to Tris-HCl buffer (50 mM with pH 8.5) in a final volume of 450 μl and preheated at 30 °C for 2 min. Then, 50 μl of 20 mM *p*NPB solution dissolved in isopropanol was added, and the reaction mixture was incubated at 30 °C for 10 min. The reaction was stopped by adding 500 μl of 5% (w/v) SDS in sodium phosphate buffer (300 mM, pH 7.0). The liberated *p*NP was spectrophotometrically quantified by measuring the absorbance at 410 nm using SpectraMAX190 Microplate Reader (Molecular Devices, Silicon Valley, CA, U.S.). One unit of enzyme activity was defined as the amount of enzyme liberating 1 μmol *p*NP per min under the above conditions. Protein concentration was determined using BCA Protein Assay Kit (Thermo Scientific Pierce, Rockford, IL, U.S.) with bovine serum albumin as standard.

2.4. Biochemical characterization of the recombinant MtCUT

The optimal pH and temperature values were determined over the range of pH 3.0-10.0 and 15–40 °C, respectively. For pH and thermal stability, MtCUT was preincubated in different pH buffers at 4 °C for 24 h or in a Tris-HCl buffer (50 mM with pH 8.5) at 25 °C, 30 °C, and 35 °C without substrate for various periods (0–90 min). The buffers used for assaying the pH optimum and pH stability were sodium citrate for pH 3.0–6.0, phosphate sodium for pH 6.0–8.0, Tris (hydroxymethyl) amino methane-HCl (Tris-HCl) for pH 8.0–9.0, and glycine-sodium hydroxide for pH 9.0–11.0. The effects of metal ions and chemical reagents were assayed under the assay conditions described above at concentrations 1 and 5 mM.

2.5. Substrate specificity and kinetic constants

Substrate specificity was determined under the assay conditions described above using the substrates *p*NPP, *p*NPB, *p*NPV, *p*NPO, and *p*NPM. The kinetic constants for the enzymes were determined under the assay conditions described above for a 5 min reaction time using *p*NPP, *p*NPB, and *p*NPV as substrates in concentration range 0–50 mM.

2.6. Degradation of cutin

Apple cutin was prepared from apple fruit as described previously [25]. For degradation of cutin, a certain amount of enzyme (1 mg) and apple cutin (100 mg) were mixed using Tris-HCl buffer (50 mM, pH 8.5) to a final volume of 10 ml. The mixture was incubated in a water bath shaker (125 rpm) for 24 h at the desired temperature. Then the supernatant was harvested by centrifugation and acidified with acetic acid. The monomers released in the supernatant were recovered by extraction with chloroform and dried using nitrogen. The dried monomers were dissolved in hexane and methyl-esterified with methanol/KOH to their corresponding methyl esters and then silylated with bis-(trimethylsilyl) trifluoroacetamide [26]. The silylated methyl esters were dissolved in hexane and analyzed using Thermo Trace1300 ISO-LT GC1300 (Thermo Fisher Scientific, Austin, TX, USA) on a DB-5MS column with temperature programmed as following: 100 °C for 3 min, 10 °C/min to 280 °C, and at 280 °C for 3 min.

2.7. Degradation of polyesters and polyvinyl acetate

The polyester films used for the enzymatic degradation were made by dissolving 1 g of raw materials in 100 ml of chloroform. The solution was poured into a polytetrafluoroethylene container and naturally dried overnight. For the degradation of polyesters, a piece of polyester film (1 × 0.5 cm^2) was placed in a reaction vial containing 1 mL Tris-HCl buffer (0.5 M, pH 8.5) and 20 μg of purified enzyme. Degradation was performed on a thermoshaker at 30 °C and 100 rpm for MtCUT. After incubation for different durations, polyester films were rinsed with deionized water and dried at 50 °C until a constant weight was obtained. The weight of the films before and after enzymatic degradation was determined and used for calculating the weight loss using the following formula:

Relative weight loss (100%) = 100 × (Initial weight–Final weight)/Initial weight

The hydrolysis of polyvinyl acetate was assayed according to the method described by Zhang et al. [23] with some modification. The

substrate solution containing 25% of polyvinyl acetate was prepared by dissolving 25 g of polyvinyl acetate in 100 ml of dimethyl sulfoxide. Then the substrate solution (0. 5 ml) and enzyme (20 μg) were added into 50 mM Tris-HCl buffer (pH 8.5) in a final volume of 1.5 ml. The reaction mixture was incubated at the desired temperature with a stirring rate of 180 rpm. The released acetic acid was quantified using the Acetic Assay Kit (Megazyme) according to the manual instructions.

2.8. Deinking experiments

The deinking process involved pulp preparation, enzymatic treatment, and flotation step. The laser paper and newspaper pulp were prepared by disintegrating 1.5 kg of paper in 15 L of tap water in a pulper. Thereafter, the pulp slurry was dehydrated, fluffed, and stored at 4 °C. Prior to the enzymatic treatment, the pulp (20 g) was suspended in 100 mM phosphate sodium buffer (pH 7.5) to a final consistency of 3% (w/v) using the high speed mixer. The enzyme (5 U/g dried pulp) and surfactant (1 mL, 0.3% AEO-9) were added to the above pulp slurry and incubated at individual optimal temperatures (37 °C and 30 °C for CrLIP and MtUT, respectively) for 3 h with gentle shaking. After the enzymatic treatment, the treated paper was subjected to the flotation step. During flotation, the released ink was continually removed away from the fiber surface. Control experiments using a heat-inactivated enzyme were also performed simultaneously. After flotation, handsheets were prepared for various TAPPI standards' testing and dirt count and brightness analysis as described previously [27]. The ink removal efficiency (%) was calculated according to Eqs. (1) and (2).

$$\text{Ink Removal Efficiency of Control Samples (\%)} = 100 \times (N_{\text{No-surfactanttreatedpaper}} - N_{\text{surfactanttreatedpaper}})/N_{\text{Nosurfactanttreatedpaper}} \quad (1)$$

$$\text{Ink Removal Efficiency of Enzyme Treated Samples (\%)} = 100 \times (N_{\text{Nosurfactanttreatedpaper}} - N_{\text{enzymetreatedpaper}})/N_{\text{Nosurfactanttreatedpaper}} \quad (2)$$

where N is the residual ink number. No surfactant-treated paper was made directly from pulp slurry without 3 h of treatment.

3. Results

3.1. Production and purification of MtCUT

A gene encoding cutinase from *M. thermophila* ATCC 42464 was selected in this study, which shows significant amino acid sequence similarity with family CE5 fungal cutinases, such as cutinase (62%) from *Colletotrichum gloeosporioides* ((accession no. AKH80819.1), cutinase (58%) from *Trichoderma harzianum* (accession no. KKP01664.1), and cutinase (56%) from *Fusarium oxysporum* Fo47 (accession no. EWZ36422.1) on CAZy database (http://www.cazy.org/http://www.cazy.org/). The gene synthesized with the optimized codon was cloned into the pPICZαA vectors and overexpressed in the heterologous host *P. pastoris* strain KM71H. The production of recombinant MtCUT was approximately 71 μg/mL in the shake flask cultures (data not shown). The recombinant MtCUT was then purified by affinity Ni-NTA Agarose resin. The theoretical molecular weight of MtCUT was 23.4 kDa. The SDS-PAGE analysis showed that the purified enzyme was approximately 23.4 kDa (Fig. 1), which is identical to theoretical molecular weight of MtCUT.

3.2. Effect of temperature, pH, and metal ions on enzyme activity and stability

The optimum temperature and pH for MtCUT were 30 °C and 8.5, respectively (Fig. 2A and B). The enzyme exhibited over 60% of relative activity from pH 3.0–11.0 and was relatively stable at a broad range between 5.0 and 9.0, retaining more than 90% of its initial activity (Fig. 2C). The enzyme exhibited over 60% of relative activity between 25 °C and 30 °C and was relatively stable at 25 °C, retaining more than

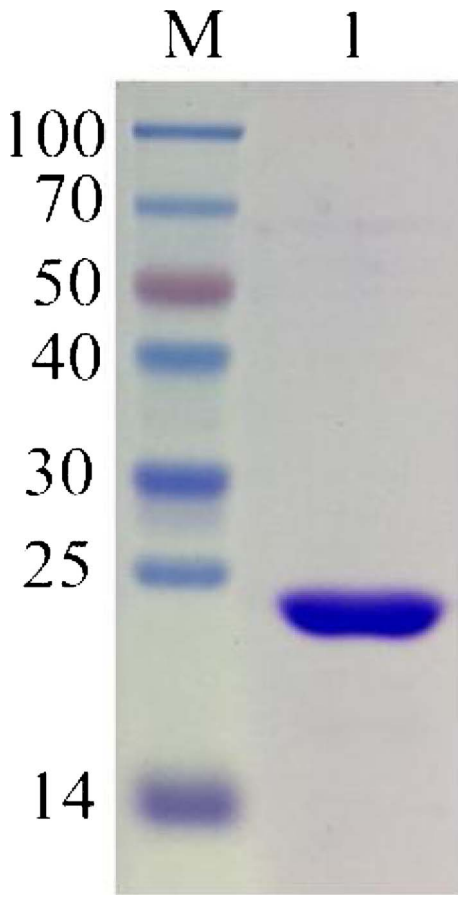

Fig. 1. SDS-PAGE of purified MtCUT. Lane M, molecular weight marker; Lane 1, MtCUT.

80% of its initial activity after 90 min of incubation. However, the remaining enzyme activity was reduced to about 60% at 35 °C (Fig. 2D). The effects of metal ions and chemical reagents on enzyme activity were tested at concentrations of 1 and 5 mM (Table 1). Cu^{2+}, Fe^{3+}, and Ni^{2+} inhibited the enzyme activity, while others had slight effects. Additionally, the MtCUT activity was strongly inhibited by SDS, which only retained 3.66% of its initial activity at concentration of 5 mM (Table 1).

3.3. Substrate specificity and kinetic constants

The specific activities of MtCUT toward different substrates were evaluated (Table 2). The enzyme catalyzed a broad range of *p*-nitrophenyl esters with C-2 to C-12 fatty acids. The highest specific activity (1059.96 U/mg) was recorded for *p*-nitrophenyl butyrate (C4). The enzyme activity decreased significantly as the carbon chain length increased above or below 4. The kinetic constants of MtCUT were tested for three substrates (Table 3). MtCUT exhibited the highest catalytic efficiency against *p*NPB.

3.4. Hydrolysis of cutin

Apple cutin was prepared and used as natural substrate to determine whether the MtCUT can indeed hydrolyze cutin. As shown in Table 4, C16 and C18 family fatty acids as cutin monomers were released after enzymatic reaction. The control experiment was also performed using heat-inactivated enzymes, in which no octadecanoic, 9-octadecenoic, and 12, 15-octadecadienoic acid were detected, except for a less amount of exadecanoic acid.

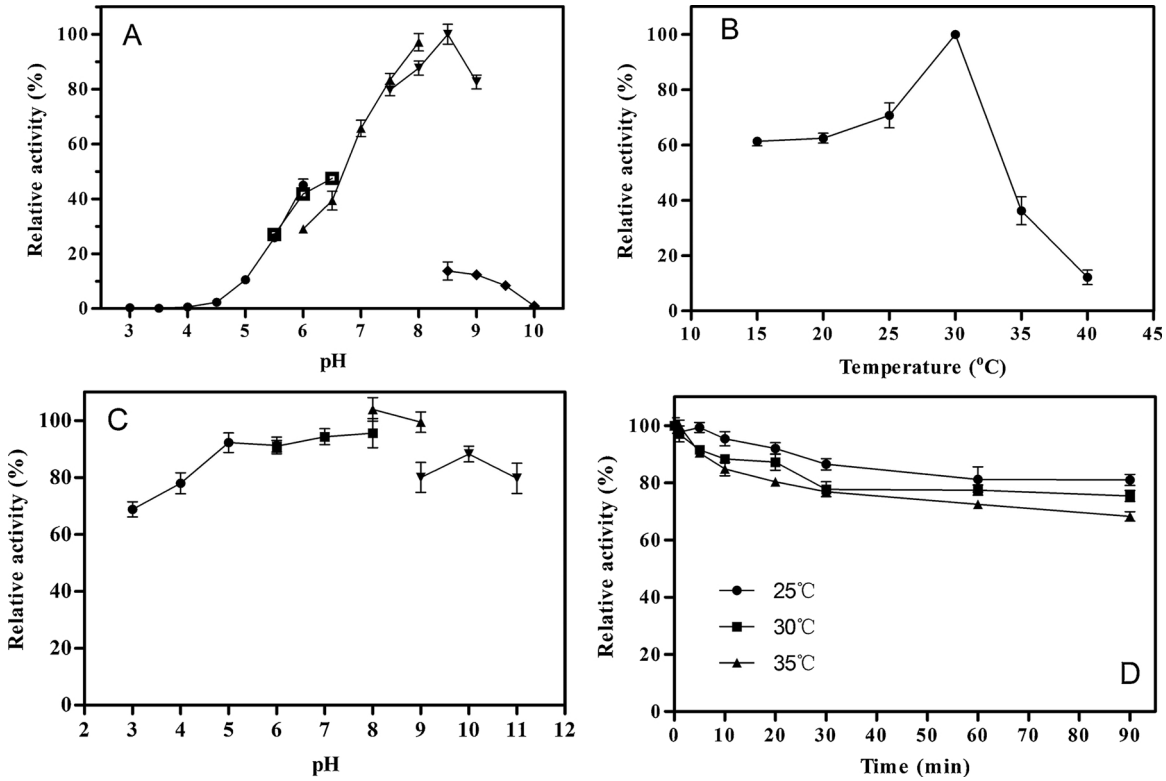

Fig. 2. Effect of temperature and pH on the activity and stability of MtCUT. A: optimum pH; B: optimum temperature: C: pH stability; D: thermal stability. The buffers used for assaying the pH optimum and pH stability were sodium citrate for pH 3.0–6.0, phosphate sodium for pH 6.0–8.0, Tris hydroxymethyl amino methane-HCl (Tris-HCl) for pH 8.0–9.0, and glycine-sodium hydroxide for pH 9.0–11.0. The buffer used for assaying the optimum temperature and stability was Tris-HCl (50 mM with pH 8.5).

Table 1
Effect of various metal ions and EDTA on MtCUT activity.

Metal ions and chemical reagents	Relative activity (%)	
	1 mM	5 mM
Co^{2+}	92.70 ± 10.37	95.87 ± 6.56
Li^{2+}	95.80 ± 2.89	102.37 ± 1.02
Zn^{2+}	101.04 ± 0.95	109.54 ± 4.72
Ni^{2+}	74.30 ± 2.95	70.09 ± 7.03
Mg^{2+}	93.17 ± 7.12	90.03 ± 5.91
Fe^{3+}	90.44 ± 0.31	74.74 ± 9.13
Na^{+}	101.63 ± 7.84	102.11 ± 7.71
Cu^{2+}	82.47 ± 1.27	68.96 ± 4.62
Ca^{2+}	94.53 ± 7.34	109.94 ± 6.20
Mn^{2+}	103.46 ± 4.10	90.32 ± 9.42
NH^{4+}	99.85 ± 7.63	92.19 ± 12.91
SDS	16.31 ± 4.29	3.66 ± 2.73
EDTA	93.99 ± 9.06	84.31 ± 0.68
Control	100 ± 2.99	

Table 2
Substrate specificity of MtCUT.

Substrates	Specific activity (U/mg)	Relative activity (%)
*p*NPA (C2)	204.93 ± 9.8	19.33 ± 0.92
*p*NPP (C3)	376.22 ± 5.36	35.49 ± 0.51
pNPB (C4)	1059.96 ± 5.22	100.00 ± 0.49
*p*NPV (C5)	566.03 ± 5.70	53.40 ± 0.54
*p*NPO (C8)	142.86 ± 9.95	13.48 ± 0.94
pNPL (C12)	125.55 ± 6.00	11.85 ± 0.57
*p*NPS (C18)	/	/

Table 3
Kinetic parameters of MtCUT.

Substrates	K_m (mM)	V_{max} (IU/mg)	V_{max}/K_m (IU/mg mM)
*p*NPP (C3)	1.41	577	409.2
pNPB (C4)	2.34	2155	920.9
*p*NPV (C5)	1.60	1240	775.0

Table 4
Monomeric products released from apple cutin hydrolyzed by MtCUT.

Cutin hydrolysis products	Area (%)	
	Control	MtCUT
Hexadecanoic acid	1.5	4.64
Octadecanoic acid	/	5.28
9-Octadecenoic acid	/	1.12
12, 15-Octadecadienoic acid	/	0.7

3.5. Degradation of polyesters and PVAc

The weight loss of polyester films following the enzymatic degradation is shown in Fig. 3A. MtCUT showed a great ability on PCL hydrolysis; a weight loss of 78.5% was obtained after 36 h of hydrolysis with MtCUT. The weight loss of PBS film reached 16.3% after 36 h of hydrolysis by MtCUT. MtCUT caused a slight weight loss on PET (5.26%), P-(3, 4) HB (3.59%), and PLA (2.22%) films. As shown in Fig. 3B, MtCUT also showed hydrolysis activity on PVAc, and the concentration of the released acetic acid reached 9.24 mg/L.

3.6. Deinking of laser-printed paper and newspaper pulp

The deinking ability of MtCUT was compared with the commercial

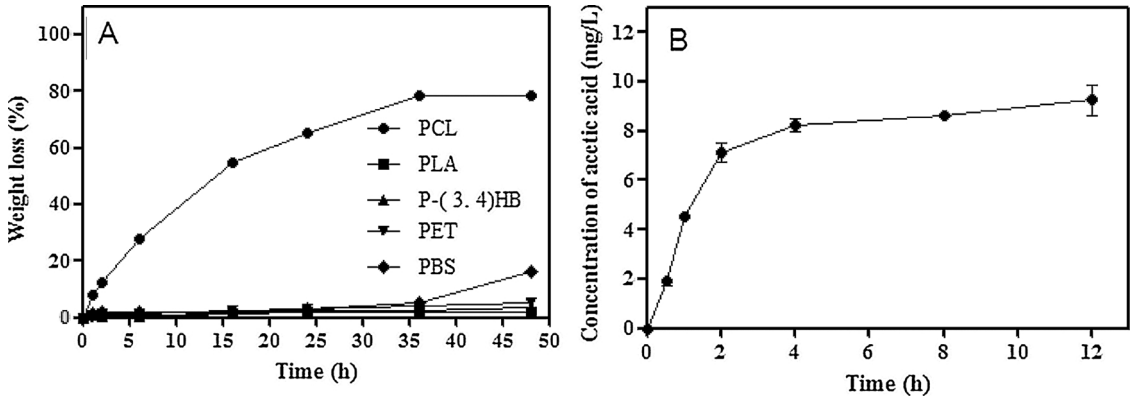

Fig. 3. Time course of the weight loss of various polyesters during degradation by MtCUT (A); time course of the concentration of released acetic acid from polyvinyl acetate during degradation by MtCUT (B).

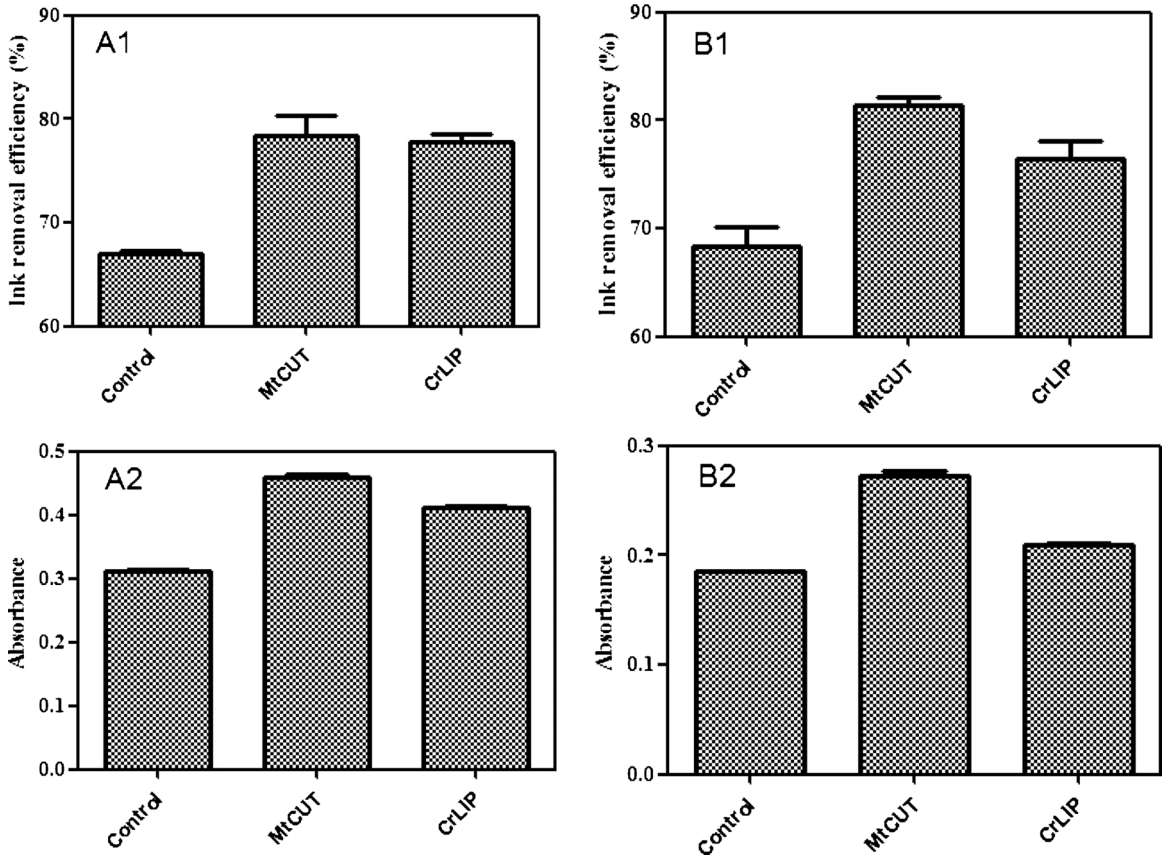

Fig. 4. Analysis of ink removal efficiency and released chromophores in the deinking effluent of laser-printed paper (A) and newspaper (B). A1 and B1: ink removal efficiency; A2 and B2: released chromophores. *Control: Heat-inactivated enzyme-treated pulp.

lipase from *C. rugosa*. As shown in Fig. 4A1 and B1, MtCUT showed better ink removal efficiency than the commercial lipase for laser-printed paper and newspaper. The brightness of MtCUT-treated papers was significantly better than that of control samples (data not shown). Cutinase or lipase treatment did not affect the paper strength properties of the pulp according to the results of tensile, tear, and burst indices compared to control samples (Fig. S1). The released chromophores in deinking effluent were analyzed in this study. MtCUT-deinked effluent samples had the highest absorbance (Fig. 4A2 and B2), which was consistent with its enzymatic deinking efficiencies. The SEM images of the deinked laser-printed paper and newspaper by MtCUT are shown in Fig. S2. After the enzymatic treatment, the fiber surface becomes smoother than that of the control samples, especially for newspaper.

4. Discussion

Unlike lipase, cutinase has the ability to hydrolyze not only soluble esters but also insoluble triglycerides and polyesters; thus, it was considered the bridge between esterase and lipase [4]. As cutinase has significant potential applications in many industrial fields, it has drawn extensive attention in recent years [3]. The thermophilic fungus *M. thermophila* ATCC 42464 is an exceptionally powerful cellulolytic organism that produces a complete set of thermal enzymes necessary for the decomposition of cellulose and hemicellulose [28,29]. In this study, we for the first time reported the characterization and deinking application of *M. thermophila* cutinase expressed in *P. pastoris* KM71H.

The molecular mass of MtCUT falls in the normal range between 22

and 26 kDa for most fungal cutinases such as cutinase from *Monilinia fructicola* [8] and TtCUT from *T. terrestris* [17] but lower than that of cutinase from *Colletotrichum kahawae* (40 kDa) [30]. Like other fungal cutinases, MtCUT has alkaline optimal pH at 8.5 [3]. The optimal temperature of MtCUT is 30 °C, which is within the range of 25 °C to 50 °C for many recombinant fungal cutinases heterologously expressed in different expression systems [31,32]. However, this value is lower than that of most of the characterized cellulases from this fungus [29]. The diversity in the temperature optima of different hydrolytic enzymes from a single fungus has been reported previously [33,34]. This may reflect the diverse function of different enzymes required for performing their catalysis efficiently in various environmental conditions.

MtCUT showed a broad range of *p*-nitrophenyl ester specificity, exhibiting activity toward *p*-nitrophenyl esters of C2 to C12 fatty acids. MtCUT displayed a better binding affinity towards *p*-NPP (C3) (K_m = 1.41 mM) and *p*NPV (C5) (1.60 mM) than toward *p*-NPB (C4) (2.34 mM), but the highest specific activity was for *p*-NPB (C4), which is in agreement with that of many cutinases from fungi such as *A. brassicicola* and *T. terrestris* [5,17]. However, its specific activity differs from that of other fungal cutinases, such as cutinases from *Sirococcus conigenus* [9], *T. harzianum* [35], *A. oryzae* [37], and *Glomerella cingulata* [32], the specificities of which were *p*NPA (C2), *p*NPV (C5), and *p*NPC (C8), respectively. The V_{max} of MtCUT is 2155 U/mg, which is much higher than that of cutinase from CUTAB1 from *A. brassicicola* [5] and *T. terrestris* [17] and Thcut1 from *T. harzianum* [35]. Biodegradable plastics such as PCL and PBS are increasingly being used in many industries as an alternative to conventional thermoplastics because of their reduced environmental impact. The degradation of such plastics by cutinases is more effective and environmentally friendly in the recycling process [36]. Similar to that of *F. solani* (30% in 6 h), the results showed that MtCUT could effectively degrade PCL; the weight loss of PCL was 27.9% in 6 h and 78.5% in 36 h. Its degradation rate was significantly higher than that of a cutinase from *Fusarium oxysporum* (6% in 18 h) [7], but lower than that of cutinase from *A. oryzae* (87% in 6 h) [37]. It was noteworthy that rapid weight loss by MtCUT occurred in the initial stage (0–24 h), which could significantly reduce the reaction time during the degradation process. Compared to PCL, the polyesters PBS, P-(3, 4) HB and PLA were relatively more difficult to be degraded by MtCUT, suggesting that MtCUT has a preference for cleaving the ester bonds of aliphatic polyesters with longer carbon chain length in monomers. Cutinases can partially hydrolyze the ester bonds on the surface of the PET fiber, but the ability greatly relies on the different nature and amount of enzymes and the crystallinity of the polymer [38]. MtCUT showed a weak hydrolysis activity on PET; further study is needed to investigate for a reliable comparison and evaluation of the catalytic activity of MtCUT on PET with other fungal cutinases.

The enzymes used for deinking now include cellulases, lipases, esterases, amylases, pectinases, and ligninolytic enzymes [21]. Most published studies on deinking deal with cellulases and lipases. Cellulases attack the fiber-ink bonding region and hydrolyze cellulose microfibrils, consequently dislodging the inks by peeling off fibers or fines on the paper surfaces. Lipases dislodged the ink particles from waste paper by directly hydrolyzing the triglycerides in vegetable oil-based inks into di- and monoglycerides and glycerol [24]. Therefore, the dirt counts and residual ink areas in the paper printed with soybean oil-based ink decreased after the deinking with a lipase from *Pseudomonas aeruginosa* [39]. Cutinases are multifunctional enzymes that can hydrolysis a variety of ester substrates, which may make them superior to lipases in waste paper deinking. In this paper, we compared the deinking capacity of MtCUT with that of a commercial lipase from *C. rugosa*. The results showed that MtCUT had higher ink removal efficiency than CrLIP, particularly for newspaper. Previous investigations reported that the enzymatic deinking can result in positive or negative effects on the physical properties of deinked paper [40]. These differential effects on deinked paper could be related to various factors including the types of enzymes and waste papers, and the dosage and treatment duration of the enzyme during enzymatic deinking [41]. In this study, after the treatment with cutinase, the physical properties of laser-printed paper and newspaper were similar or even slightly increased. The SEM images showed that the fiber surface of the laser-printed paper and newspaper became smoother, and this may be correlated with the degradation of the sticky deposits, such as polyvinyl acetate. All the results revealed that MtCUT has a great potential in deinking process.

In conclusion, we successfully expressed a cutinase from *M. thermophila* in the heterologous host *P. pastoris* KM71H and characterized it. Moreover, we demonstrated that MtCUT has a great potential in PCL hydrolysis and deinking process at a mild temperature (30 °C), which could simplify the deinking process and reduce the energy. Our research may provide a new potential application field for cutinases. Future research will focus on the combined effect of cutinases and cellulases or other enzymes in waste paper deinking.

Conflict of interest

None.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.procbio.2017.11.021.

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