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Research Article

Purification and Characterization of Poly(3-Hydroxybutyrate-*co*-3-Hydroxyvalerate) Depolymerase from Thermophilic *Actinomadura* sp. TF1

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ABSTRACT

Actinomadura sp. strain TF1, a thermophilic actinomycete bacterium that produces an enzyme capable of degrading the bioplastic copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), was cultured in basal medium under optimized conditions at pH 8, 45 °C, and 0.4% (w/v) PHBV substrate concentration, as previously studied, to achieve the highest production of PHBV depolymerase. The enzyme was purified through 80% (w/v) ammonium sulphate precipitation, dialysis, and Diethylaminoethyl cellulose (DEAE) column chromatography, resulting in a specific activity of 463.7 U/mg protein, 49.9% recovery, and a 2.8-fold purification. The molecular weight of the purified PHBV depolymerase was determined to be 35 kDa by SDS-PAGE. The enzyme exhibited optimal activity at 45 °C and demonstrated thermal stability within the range of 35-45 °C. The optimal pH for activity was 8, with stability observed between pH 8 and 9. PHBV depolymerase displayed strong activity against substrates such as PHBV, polyhydroxybutyrate (PHB), and polybutylene succinate (PBS), with the highest degradation activity observed for PHBV. Additionally, the enzyme hydrolyzed *p*-nitrophenol esters, confirming its classification as an esterase. The activity of PHBV depolymerase was enhanced by the presence of Ca^{2+}, Na^{+}, and K^{+} ions but was inhibited by Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Hg^{2+} ions. Dithiothreitol (DTT) exhibited a significant inhibitory effect. In conclusion, this study is the first to report the purification and characterization of PHBV depolymerase from *Actinomadura* sp. strain TF1.

Keywords: Polyhydroxybutyrate-*co*-polyhydroxyvalerate, PHBV depolymerase, Bioplastics, *Actinomadura* sp., Polyhydroxyalkanoates

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Introduction

Plastics, semisynthetic organic polymers composed of high-molecular-weight chain-like molecules are major contributors to environmental pollution [1]. Since 2020, the United Nations has reported annual plastic waste generation in excess of 400 million metric tons. By 2035, waste production is projected to double to 800 million metric tons, potentially reaching 1,600 million metric tons by 2050 [2]. To mitigate environmental impact, using biodegradable plastics such as polycaprolactone (PCL), polylactic acid (PLA), polybutylene succinate (PBS), polybutylene succinate-*co*-butylene adipate (PBSA), polybutylate adipate terephthalate (PBAT), and polyhydroxyalkanoates (PHAs) as substitutes for synthetic plastics presents a promising approach for the development of alternative biomaterials.

PHAs, microbiologically produced biodegradable polyesters, serve as intracellular carbon and energy reserves under nutrient-limiting conditions (e.g., nitrogen or phosphorus) in the presence of excess carbon [3]. Their physical and chemical characteristics are comparable to those of synthetic polymers including polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), and low-density polyethylene (LDPE). These properties are influenced by factors including microorganism type, carbon source, fermentation conditions, and recovery technologies [4]. Unlike petroleum-based polymers, PHAs are a class of biodegradable polymers produced via microbial fermentation of renewable resources. As fully biosynthesized and biodegradable materials, PHAs represent a promising solution to mitigate environmental concerns. Among the various types of PHAs, polyhydroxybutyrate (PHB) and poly(3-hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) are the most widely studied and prominent. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a copolymer of hydroxybutyrate (HB) and hydroxyvalerate (HV), accumulates in bacteria when organic acids are present in the growth media. PHBV is stronger and more flexible than PHB due to its lower melting temperature and reduced crystallinity. It also exhibits enhanced physical properties such as impact resistance, durability, and flexibility, making it suitable for industrial applications. PHAs are degraded by diverse microorganisms, including Gram-positive and Gram-negative bacteria, actinomycetes, and fungi, in both terrestrial and aquatic environments [5]. The degradation of PHAs involves extracellular PHB depolymerases, which hydrolyse PHB into water-soluble products that then serve as carbon and energy sources. Many PHB depolymerases have previously been purified and characterized [6, 7]. PHBV is degraded under natural conditions by PHBV depolymerases, which are produced by a wide variety of microorganisms. *Actinomadura*, a genus of thermophilic actinobacteria, is known for producing extracellular enzymes and degrading PHBV [5]. While, studies on the purification and characterization of thermophilic PHBV depolymerase from *Actinomadura* are limited, some reports have focused on isolating and characterizing thermophilic PHBV depolymerases from Gram-positive actinomycetes, particularly *Actinomadura* sp. AF-555 [8]. In a previous study, we optimized conditions to maximize PHBV depolymerase production by enhancing bacterial growth in *Actinomadura* sp. strain TF1 [9]. Building on these findings, this study details the purification and characterization of a PHBV-degrading enzyme for potential biological applications.

Materials and methods

Substrates and chemicals

PHBV (containing 12% 3-hydroxyvalerate (3HV)), PHB, PBS, PLA (*Mn* 5.4×10^3), polylactic-L-acid (PLLA), and *p*-nitrophenol ester substrates were purchased from Sigma-Aldrich Chemical (USA), while all remaining chemicals utilized in the study were of analytical grade.

Growth conditions and PHBV depolymerase enzyme assay

For the preparation of the inoculum, *Actinomadura* sp. strain TF1 was cultured in 100 mL of low-salt Luria-Bertani broth (LB_{low}), consisting of 10 g/L peptone, 5 g/L NaCl, and 5 g/L yeast extract in 250 mL Erlenmeyer flasks. The culture was incubated at 45°C on a rotary shaker (180 rpm) for 96 h. Subsequently, the cell pellet was collected through centrifugation at 9,100 × *g* for 15 minutes at 4°C and washed once with basal media. The basal media was comprised of 1.6 g/L K_2HPO_4, 1 g/L $(NH_4)_2SO_4$, 200 mg/L yeast extract, 200 mg/L $MgSO_4 \cdot 7H_2O$, 200 mg/L KH_2PO_4, 100 mg/L NaCl, 20 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L $FeSO_4 \cdot 7H_2O$, 0.5 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg/L $MnSO_4$, and 0.5 mg/L $Na_2WO_4 \cdot 2H_2O$ at pH 7. TF1 cells were cultivated in 100 ml of basal media containing 0.4% (w/v) PHBV in 250 ml Erlenmeyer flasks to product PHBV-degrading enzymes. These cultures were incubated at 45 °C, on a shaker at 180 rpm for 144 h under optimal conditions [9]. PHBV depolymerase activity was measured via a slightly modified method derived from Kobayashi et al. [10]. In this process, PHBV (0.1% w/v) was suspended in 100 mM Tris-HCl buffer at pH 8. The substrate suspension was subjected to sonication for 20 minutes using an ultrasonic processor. Subsequently, 0.5 mL of the culture supernatant was added to 2.5 mL of the substrate suspension and incubated at 45°C for 24 h. After 24 h, the turbidity resulting from insoluble plastics was measured at 650 nm. One unit (U) of polyester depolymerase activity was defined as the amount of enzyme required to reduce the turbidity of the polyester substrate by 0.1 at an optical density (OD_{650} nm) under the specified experimental conditions.

Purification of PHBV depolymerase

Actinomadura sp. TF1 was cultured in PHBV basal media for 144 h at 45 °C. Following cultivation, the culture media was centrifuged at 9,100 × *g* for 15 minutes at 4 °C to separate the supernatant, which was subsequently employed for enzyme preparation. The enzyme purification procedures were carried out at 4 °C. Initially, the crude enzyme was precipitated using ammonium sulphate (80% saturation) with continuous stirring for 6 h, after which it was centrifuged at 9,100 × *g* for 30 minutes. The resulting precipitate was dissolved in 50 mM Tris-HCl buffer (pH 8), transferred to a dialysis bag, and incubated overnight for dialysis in the same chilled buffer. The dialyzed enzyme was then concentrated using a Vivaspin 20 concentrator equipped with a 10 kDa molecular weight cut-off membrane filter (Sartorius, Tokyo, Japan). The concentrated protein was subsequently subjected to purification through diethylaminoethanol (DEAE)-Sepharose (DEAE Sepharose Fast Flow, GE Healthcare) ion-exchange chromatography. A column measuring 2.5 cm × 20 cm was manually packed and equilibrated with the dialysis buffer. Proteins were eluted by applying a linear gradient of 0 to

500 mM NaCl in a 50 mM Tris-HCl buffer (pH 8) at a flow rate of 1 mL/minute. The purified PHBV depolymerase fractions were collected and stored at -20 °C until further use.

Protein measurement

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to assess the purified PHBV depolymerase on a 12.5% polyacrylamide gel. In order to determine the molecular mass of the enzymes, Coomassie brilliant blue R-250 was used to stain the gel, allowing for the visualization of protein bands. For zymography, the SDS-PAGE gel was subjected to a process to remove sodium dodecyl sulfate. This involved gently shaking the gel in 25% (v/v) isopropanol at 4 °C for 1 h, followed by two subsequent washes with distilled water to eliminate the isopropanol. After washing, the gel was overlaid with a 0.04% PHBV agar gel containing 50 mM Tris–HCl buffer (pH 8) and maintained at 45 °C for 3 h. A clear zone on the white background of the gel indicated activity in a resolved band, as described by Sriyapai et al. (2015) [11]. Protein concentrations were measured using the protein assay reagent (Bio-Rad) using BSA as the standard, as described by Bradford (1976) [12].

Effects of pH and temperature on PHBV depolymerase and stability

The optimum pH was measured using three different buffers at a final concentration of 50 mM with a pH range of 3-12 (acetate buffer for pH 3-6, phosphate buffer for pH 6-8, Tris–HCl buffer for pH 8-10, and glycine-NaOH buffer for pH 10-12) at 45 °C for 120 minutes. The optimum temperature was determined in 50 mM Tris–HCl buffer (pH 8) at varying temperatures within the range of 35 to 95 °C. For the pH stability was determined by measuring the residual activity after incubating the enzyme at various pH values at 4 °C for 24 h. Thermostability, the purified enzyme was incubated in 50 mM Tris–HCl buffer (pH 8) for 180 minutes at temperatures ranging from 35 to 75 °C. All of the assays were performed in triplicate.

Substrate specificity

The enzymatic activity of purified PHBV depolymerase on PHBV, PHB, PBS, PLA, PLLA, and polycaprolactone (PCL) was examined in experiments involving the degradation of these polyesters, following the previously described procedure.

Protease activity was determined by using casein as a substrate, with slight modifications to the method described by Jeong et al. (2018) [13]. The reaction mixture consisted of 2.5 mL of a 0.6% (w/v) casein solution in 50 mM Tris-HCl buffer (pH 8) and 0.5 mL of the purified enzyme solution. This mixture was incubated at 45 °C for 10 minutes. The reaction was then terminated by the addition of 5 mL of 0.3 M trichloroacetic acid. The entire mixture was then centrifuged at 10,000 × *g* for 10 minutes at 4°C, and the supernatant (2 mL) was added to 5 mL of 0.5 M Na_2CO_3 solution, followed by 1 mL of 0.5 M Folin-Ciocalteu reagent, which was then mixed thoroughly. The color generated after 30 minutes of incubation at 37 °C was filtered and then measured at 660 nm via a UV-visible spectrometer. One unit of protease activity was defined as the amount of the enzyme that produced the

equivalent of 1 μg of tyrosine per minute under the assay conditions (pH 8, 45°C). All of the assays were carried out in triplicate.

Esterase activity was assessed at 410 nm using various *p*-nitrophenol esters with differing acyl chains, ranging from *p*-nitrophenyl-acetate (C2) to *p*-nitrophenyl-octanoate (C8), following the method outlined by Sriyapai et al. (2015) [11]. The enzyme reaction was conducted for 5 minutes at 45 °C in 50 mM Tris–HCl buffer (pH 8) containing 0.4% Triton X-100 and 0.1% gum arabic. To stop the reaction, a final concentration of 0.2% (w/v) SDS was added, and the mixture was placed on ice. One unit of esterase activity was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol per 1 minute at 45 °C. The extinction coefficients of *p*-nitrophenol were determined for every condition prior to each measurement. Background substrate hydrolysis was measured in a reference sample lacking the enzyme. All of the experiments were conducted in triplicate.

Effects of metal ions, surfactants and inhibitors

The effect of metal ions on enzyme activity was evaluated by incubating the purified enzyme with different concentrations of metal ions (1 mM and 5 mM) including Ca^{2+}, Na^{+}, K^{+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Hg^{2+}. The influence of surfactants (0.1% and 0.5%) (β-mercaptoethanol, Triton X-100, Tween 80, sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB)) on the activity of purified enzyme was investigated. The effect of inhibitors, the potential inhibitors used were phenylmethylsulfonyl fluoride (PMSF) and dithiothreitol (DTT), and residual activity was determined by incubating the purified enzyme at various concentrations (1 mM and 5 mM). The enzyme activity was measured for 1 h at 45 °C. The reaction mixture without metal ions, surfactants and inhibitors was used as a control. All reactions were carried out in triplicate.

Results and Discussion

Purification of PHBV depolymerase

Compared with PHB, PHBV has several significant advantages in terms of thermomechanical properties, including greater ductility and flexibility, lower crystallinity, improved tensile strength, and lower melting point [14]. The degradation of PHBV in the environment has been extensively studied. Several PHBV-degrading bacteria have been identified, including *Bacillus* sp. AF3, *Acidovorax* sp. HB01 and *Pseudomonas mendocina* [5, 15, 16]. Among actinomycetes, *Actinomadura* sp. AF-555, *Streptomyces* sp. IN1, and *Streptomyces* sp. AF-111 have been identified as PHBV-degrading [8, 17, 18]. Additionally, a novel thermophilic *Actinomadura* sp. strain TF1 was found to be capable of degrading biodegradable polyesters such as PLA, PLLA, PBS, PBSA, and PCL, in addition to PBS films in basal media and PHBV films after burial in soil [9, 19, 20]. This strain exhibited the highest PHBV depolymerase activity when cultured in PHBV basal media under the optimal enzyme production conditions of pH 8 and 45°C at a 0.4% (w/v) PHBV substrate concentration [9]. PHBV depolymerase was purified from the culture supernatant of *Actinomadura* sp. TF1 and precipitated at 80% ammonium sulphate saturation, resulting in a 1.3-fold purification with a recovery percentage of 71.4%. Using a

DEAE-Sepharose chromatographic column, the purification efficiency and yield were 2.8-fold and 49.9%, respectively. The purification process is summarized in Table 1. SDS-PAGE and zymography revealed a single band, indicating homogeneous PHBV depolymerase activity. The molecular mass of the purified enzyme was determined to be approximately 35 kDa, and the enzyme was able to degrade PHBV as a substrate (Figures 1A and 1B). This molecular weight is consistent with those of PHBV depolymerases from *Bacillus* sp. AF3 (37 kDa) [5] and *Streptoverticillium kashmirense* AF1 (37 and 45 kDa) [21]. To our knowledge, this is the first report describing the characterization of a purified PHBV depolymerase from *Actinomadura* sp. The degradation of 0.1% (w/v) bioplastic emulsions (PHBV, PHB, and PBS) in 50 mM Tris-HCl buffer (pH 8) on agar plates by purified PHBV depolymerase at 45°C for 24 h is shown in Figure 1C. The purified enzyme degraded PHBV and PHB more effectively than it did PBS. This observation was consistent with the findings of Allen et al. (2011) [17], who reported that PHBV depolymerase activity from *Streptomyces* sp. IN1 could degrade both PHBV and PHB on agar media, but that the enzyme activity decreased when PHB was degraded, indicating that this enzyme had greater specific activity for PHBV than for PHB.

Effects of pH and temperature on the PHBV-degrading activity of the purified enzyme

The activity of PHBV depolymerase was assayed with variations in pH and temperature (conditions described in the Materials and Methods). The maximum enzyme activity was observed at 45 °C and pH 8 for 120 minutes (Figures 2A and 2B). The enzyme was highly stable within a pH range of 8 to 10 and retained more than 80% of its activity within a pH range of 7 to 10 at 4 °C for 24 h (Figure 2C). The thermostability of the purified enzyme was detected from 35 °C to 75 °C for 180 minutes. The enzyme retained 90% of its activity at 35 °C and 45 °C for 120 minutes and maintained over 80% activity at 55 °C for 60 minutes (Figure 2D). The decrease in enzyme activity with increasing temperature reflects the thermolabile nature of the enzyme [7]. Previous studies have reported that the extracellular PHB/PHBV depolymerases from *Acidovorax* sp. HB01, *Pseudomonas mendocina*, *Streptoverticillium kashmirense* AF1, and *Streptomyces* sp. MG exhibited optimal temperature and pH ranges of 45-60°C and 8-8.5, respectively [15, 16, 21, 22]. Additionally, the optimal pH and temperature of the PHB depolymerase from fungi were found to be in the range of 45-55 °C and 5-7, respectively, depending upon the strain, such as *Aspergillus* sp. NA-25, *Fusarium solani* Thom, and *Penicillium expansum* [23-25].

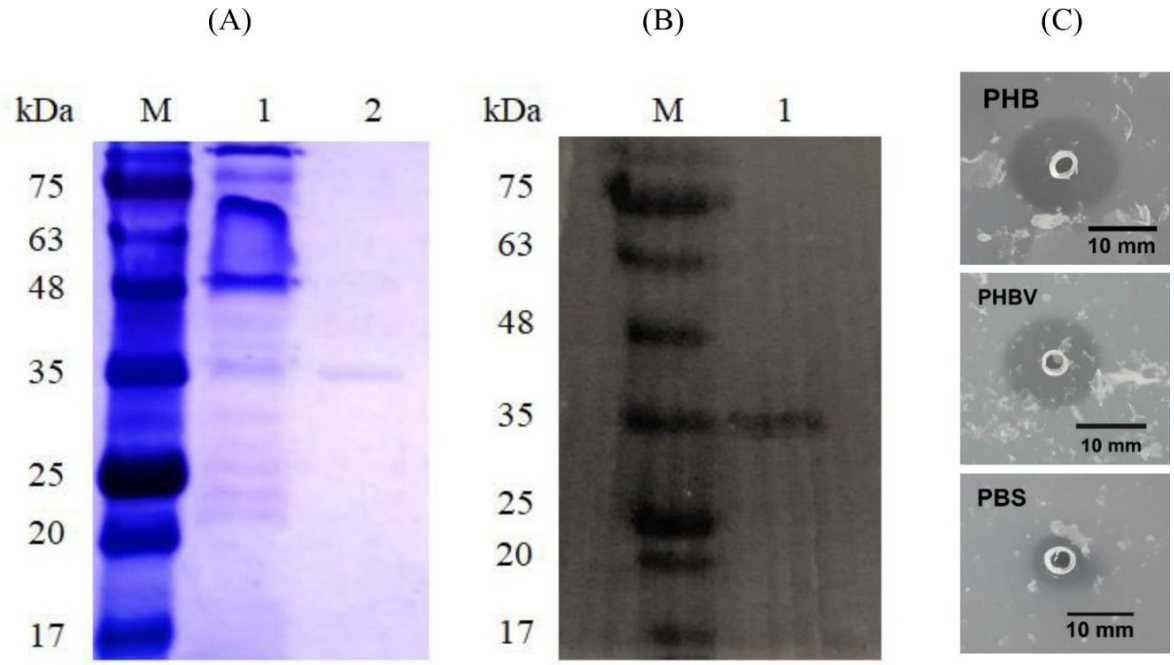

Figure 1 The purified PHBV depolymerase enzyme from *Actinomadura* sp. TF1. A: SDS-PAGE. Lane M: molecular weight standards; Lane 1: 80% ammonium sulfate fraction after dialysis; Lane 2: the purified PHBV depolymerase eluted from DEAE-Sepharose. B: zymography. Lane M: molecular weight standards; Lane 1: the purified PHBV depolymerase. C: the degradation of 0.1% (w/v) bioplastic in 50 mM Tris-HCL, pH 8.0 buffer with purified PHBV depolymerase at 45 °C for 240 minutes of incubation.

Table 1 Summary of purification of PHBV-degrading enzyme from *Actinomadura* sp. TF1.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purification fold
Culture supernatant	19.7	3,271	165.7	100	1
$(NH_4)_2SO_4$ (80%)	10.7	2,334	218.5	71.4	1.3
DEAE-Sepharose	3.5	1,630	463.7	49.9	2.8

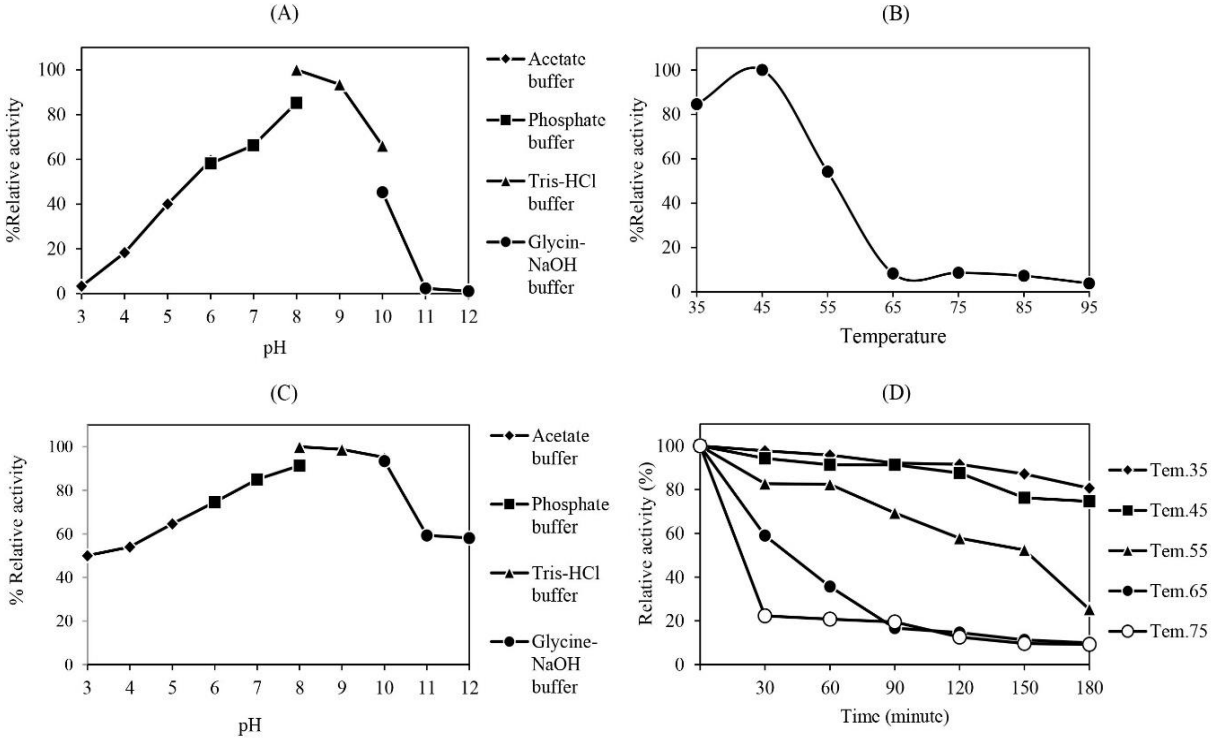

Figure 2 Effects of pH and temperature on the PHBV-degrading activity of the purified PHBV depolymerase. A: optimal pH. B: optimal temperature. C: stability of the PHBV depolymerase at different pH. D: stability of the PHBV depolymerase at different temperature. The PHBV-degrading activity obtained under standard conditions was defined as 100 %.

Substrate specificity of the PHBV depolymerase

The hydrolytic activities of the purified PHBV depolymerase on various substrates were investigated (Table 2). The enzyme degraded PHBV, PHB, and PBS, but exhibited no hydrolytic activity against PLA, PLLA or PCL. The enzyme showed the highest activity against PHBV, with specific activities of 386.54 ± 8.61 U/mg, 210.83 ± 6.75 U/mg, and 86.67 ± 4.86 U/mg, for PHBV, PHB, and PBS, respectively, indicating a narrow substrate range focused on specific PHAs. The enzyme catalyses the hydrolysis of ester bonds in PHB and PHBV, producing β-hydroxybutyrate monomers as the primary degradation products. Consistent with previous reports, the PHBV depolymerase from *Acidovorax* sp. HB01 showed greater activity against PHBV than against PHB, P(3HB-co-4HB), or PCL, supporting its specificity for PHBV [15]. The enzyme also demonstrated catalytic activity towards various *p*-nitrophenyl-acyl ester substrates but showed no activity with casein, indicating its specificity for ester bond hydrolysis in PHB and PHBV as well as its true depolymerase nature. These results align with findings on PHB depolymerase from *Fusarium solani Thom*, which exhibited specificity towards PHBV, but not PCL and possessed esterase activity, as demonstrated by its activity towards other *p*-nitrophenyl alkanoates, such as *p*-nitrophenyl acetate and *p*-nitrophenyl butyrate [24].

Table 2 Substrates specificity of the purified PHBV depolymerase.

Substrate	Specific activity (U/mg protein)
polyester*	
PHBV	386.54±8.61
PHB	210.83±6.75
PBS	86.67±4.86
PLA	0
PLLA	0
PCL	0
protein	
casein	0
p-nitrophenol ester (3 mM)	
*p*NP-acetate (C2)	48.13±3.11
*p*NP-butyrate (C4)	27.97±1.21
*p*NP-octanoate (C8)	2.12±4.23

*Concentration of each polyester is 0.1% (w/v)

Effect of metal ions on the activity PHBV depolymerase

The effects of metal ions on enzyme activity were evaluated by incubating the purified enzyme with various metal ions at concentrations of 1 mM and 5 mM for 1 h at 45°C (Table 3). The addition of Ca^{2+}, Na^{+} and K^{+} significantly increased the hydrolysis rate, whereas the addition of Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and Hg^{2+} inhibited enzyme activity. These findings indicate that PHBV depolymerase activity is markedly influenced by metal ions, which can either enhance or inhibit its function. This observation is consistent with the findings of Wang et al. (2015) [15], who reported increased hydrolysis rates for PHBV depolymerase from *Acidovorax* sp. HB01 in the presence of Na^{+}, K^{+}, and Ca^{2+}, whereas Zn^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} inhibited activity. Similarly, Sayyed et al (2019) [7] reported enhanced activity of PHB depolymerase from *Microbacterium paraoxydans* RZS6 in the presence of Ca^{2+} and Mg^{2+}, but activity was inhibited by Fe^{2+} at 1 mM. Calcium ions (Ca^{2+}) were found to be essential as cofactors for optimal enzyme function, with concentrations between 1 and 100 mM significantly enhancing activity. However, high concentrations of Na^{+} and K^{+} have been reported to inhibit activity [26]. Shah et al. (2008) [21] reported that PHBV depolymerase activity from *Streptoverticillium kashmirense* AF1 was optimal at pH 7–8 and 45°C, with Ca^{2+} enhancing activity and Fe^{2+} reducing it. The inhibitory effects of Fe^{2+}, Zn^{2+}, and Cu^{2+} highlight the need for careful control of metal ion concentrations to avoid hindering enzymatic performance.

Table 3 Effect of metal ions on the activity of purified PHBV depolymerase.

Metal ion	Concentration (mM)	%Relative activity
No metal ion		100
Ca^{2+}	1	126.1±0.10
	5	112.3±0.15
Na^{+}	1	109.8±0.08
	5	100.3±0.23
K^{+}	1	111.6±0.12
	5	105.8±0.13
Fe^{2+}	1	33.7±0.68
	5	12.7±0.24
Mg^{2+}	1	91.6±0.46
	5	84.3±0.37
Mn^{2+}	1	81.1±0.27
	5	58.4±0.34
Zn^{2+}	1	35.6±0.18
	5	22.4±0.81
Cu^{2+}	1	61.4±0.14
	5	14.2±0.74
Hg^{2+}	1	58.8±0.38
	5	38.6±0.27

Effect of the reagents on the activity of PHBV depolymerase

The effects of various reagents on the activity of the purified enzyme were determined by incubating the enzyme with differing concentrations of each reagent (Table 4). The activity of PHBV depolymerase was significantly influenced by β-mercaptoethanol, Triton X-100, Tween 80, SDS, CTAB, PMSF, and DTT. As the concentration of these reagents increased, a reduction in PHBV depolymerase activity was observed, indicating that the inhibitory effects of the reagents are concentration dependent. The enzyme was strongly inhibited by the sulfhydryl-reducing agents DTT and β-mercaptoethanol, which is consistent with previous reports indicating that DTT completely inhibits enzyme activity at concentrations as low as 1 mM, likely by disrupting essential disulfide bonds, critical for maintaining the enzyme's native structure and catalytic function [27]. Similarly, Wani et al. (2016) [28] reported that β-mercaptoethanol caused a maximum inhibition of 85% in purified PHB depolymerase from *Stenotrophomonas* sp. RZS 7, suggesting that it interferes with the active site or overall structure of the enzyme due to its reducing properties, which disrupts essential disulfide bonds. Tween 80 strongly inhibited PHBV depolymerase activity, more than Triton X-100 did. Both nonionic detergents are widely

used as plasticizers in the manufacture of PHBV products. Tween 80 was found to reduce PHBV depolymerase activity to 24% in *Streptomyces* sp. SSM 5670, suggesting that it may interact with hydrophobic regions of the enzyme, potentially near to or at the active site, thereby altering its conformation or affecting substrate binding [29, 30]. Ionic detergents such as CTAB and SDS also inhibited enzyme activity, which is consistent with the findings of Bhatt et al. (2010) [31], who reported that 0.01% (w/v) CTAB and SDS caused 19% and 33% inhibition, respectively, of PHB depolymerase activity in *Aspergillus fumigatus* 202. However, higher concentrations of these detergents significantly inhibited enzyme activity. PMSF, a well-known serine protease inhibitor, specifically targets serine residues in enzymes, which are often crucial for their catalytic activity. PHBV depolymerases from strain TF1 were inhibited in the presence of PMSF, indicating that serine was present in their active sites. This observation aligns with the findings of Nadhman et al. (2015) [23], who reported that four types of purified PHB depolymerases from *Aspergillus* sp. strain NA-25 were related to the serine group of hydrolases, as indicated by their sensitivity to serine protease inhibitors.

Table 4 Effect of reagents on the activity of purified PHBV depolymerase.

Reagent	Concentration	%Relative activity
surfactants No reagent		100
β-mercaptoethanol	0.1%	55.9±0.77
	0.5%	22.5±0.91
Triton X-100	0.1%	94.7±0.16
	0.5%	93.3±0.01
Tween 80	0.1%	66.5±0.87
	0.5%	37.7±0.98
SDS	0.1%	85.5±0.25
	0.5%	33.5±0.50
CTAB	0.1%	35.7±0.39
	0.5%	16.5±0.92
inhibitors PMSF	1 mM	83.7±0.51
	5 mM	71.3±0.32
	10 mM	52.2±1.77
DTT	1 mM	53.7±0.18
	5 mM	13.5±0.36
	10 mM	8.3±0.21

Conclusions

In this study, a novel extracellular PHBV depolymerase from *Actinomadura* sp. TF1 was identified and successfully purified. The biochemical properties of the purified enzyme, including its molecular mass, optimal temperature, optimal pH, and sensitivity to chemical inhibitors, were characterized. These findings indicate that the enzyme can depolymerize a broad range of polyester-based biodegradable polymers, highlighting its potential for sustainable plastic waste management. Large-scale production of the enzyme is now being carried out through molecular cloning and fermentation studies, facilitating further characterization and application in plastic biodegradation.

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