

# Purification, Characterization and De-Staining Potentials of a Thermotolerant Protease Produced by *Fusarium oxysporum*

Mohammed Inuwa Ja'afaru<sup>1</sup>, Konjerimam Ishaku Chimbekujwo<sup>1</sup>, Obinna Markraphael Ajunwa<sup>1\*</sup>

<sup>1</sup> Department of Microbiology, School of Life Sciences, Modibbo Adama University of Technology, P.M.B. 2076, Yola, Adamawa State, Nigeria

\* Corresponding author, e-mail: [obinna.ajunwa@mautech.edu.ng](mailto:obinna.ajunwa@mautech.edu.ng)

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## Abstract

Proteases are important industrial enzymes and fungi prove to be good sources of such enzymes. Purification techniques are however necessary for increased specificity in activity and better industrial value. Based on this, a protease produced by a *Fusarium oxysporum* was purified to homogeneity by Sephadex G-200 column and  $\alpha$ -casein agarose chromatography. The enzyme had a molecular weight of 70 kDa in SDS-PAGE. Purified *Fusarium oxysporum* protease had a specific activity of 93.88 U/mg protein. The purification magnitude was 7.7 and the total yield was 20 %. Purified protease had an optimum pH of 5.0 while the optimum temperature was 40 °C. The enzyme was also thermotolerant (approximately 100 % at 40 °C for 2 h). The enzyme activity was stimulated by surfactants and metal ions like, Tween-20 and Mg<sup>2+</sup>. Enzyme activity was inhibited in presence of PMSF and EDTA. Casein was found to be the best substrate for protease activity of *Fusarium oxysporum* FWT1. Protease were tested upon blood stain for de-clotting of blood and was found to exhibit good de-clotting and de-staining activity after 15 minutes treatment time.

## Keywords

*Fusarium oxysporum*, protease, enzyme purification, thermotolerant

## 1 Introduction

Proteases are degradative enzymes, which catalyze the total hydrolysis of proteins. Microbial proteases are proteases from microorganisms and are very important in the global enzyme trade, as they account for up to 60 % of the total enzyme merchandise [1]. Proteases as important industrial enzymes can be incorporated in detergents, therapeutics, food industries, leather, meat processing and cheese making [2-4]. Considering the industrial importance of microbial enzymes and to fulfill the constant need of proteases, new enzymes for use in commercial applications with desirable biochemical and physiochemical characteristics and low production cost have been the focus of much research [2, 5].

Fungi produce a wide variety of proteolytic enzymes depending on the specie and the environment, with filamentous fungi having potentials to grow and produce enzymes under varying environmental and physiological conditions [6]. Some of these factors such as production time course, metallic ions, surfactants, inhibitors, pH and temperature, utilizing a wide variety of substrates as nutrients are important in characterization of fungal proteases [2]. However, the

major criteria for proteases selection are based on the pH activity as well as their thermal stability [2, 7]. Fungal proteases have been a major important commodity in industrial enzyme production and have been applied hydrolytically on a variety of proteinaceous systems [7, 8].

As a key factor of bioresource sustainability, fungal proteases have been accorded some major regard because of the ability of fungi to proliferate on inexpensive substrates while excreting copious amounts of enzymes into the surrounding nutrient medium [8]. The efficiency of fungal protease production as well as its catalytic properties have been identified to be specie specific as fungal genera like *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* have been identified as good sources of proteases [8]. A report by [9] showed that *Fusarium oxysporum* was implicated as one of the abundant fungal species with a good proteolytic potential, with enzymes from *F. oxysporum* strains showing a good deal of thermotolerance [7, 9]. *F. oxysporum* proteases have also been proven to catalytically attack a variety of unique substrates with proteinaceous components like rice bran and chitin with a high efficiency level

[9, 10], thereby making them microorganisms of choice in protease production.

The present study focused on purification, characterization and application of the protease produced by a *Fusarium oxysporum* isolate using wheat bran as substrate, with the possibility of opening up a path in its industrial scale production and application.

## 2 Materials and methods

### 2.1 Microorganism

The test microorganism was a thermotolerant protease producing fungal isolate coded as FWT1, which was earlier isolated from a refuse waste dump site in Jimeta, Yola, Nigeria (9.2035° N, 12.4954° E). Following molecular characterization using ITS sequence, the fungi was identified as a *Fusarium oxysporum* FWT1 (NCBI accession number MF599163.1). The strain is stored in the culture bank of the Department of Microbiology, Modibbo Adama University of Technology.

### 2.2 Protease production

Ten grams of substrate (wheat bran) was moistened with salt solution [composed of (% w/v):  $\text{NH}_4\text{NO}_3$  0.5, potassium dihydrogen orthophosphate 0.2,  $\text{NaCl}$  0.1 and  $\text{MgSO}_4$  0.1], with trace elements:  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.01 ml of each and sterilized at 121.5 °C for 15 min, cooled, inoculated with 1 ml of  $10^6$  spore suspension of *F. oxysporum* and incubated at 28 °C for 7 days [11].

### 2.3 Enzyme extraction

One milliliter of Tween 80 was added in 100 ml of distilled water. 100 ml of the solution was added into the fermented substrate and was homogenized on a rotary shaker at 150 rpm for 1 hour. The solution was then filtered and the solids were removed by centrifuging the homogenate at 3,500 x g for 15 mins at 4 °C. The resultant supernatant was used for analytical studies [11].

### 2.4 Assay of protease activity

Total protease activity in culture supernatant was measured according to the procedure stated by Keay and Wildi [12] with minor modifications using casein as substrate. One milliliter of 1 % (w/v) casein in 0.1 M sodium phosphate buffer (pH 7.0) was mixed with 1 ml of culture supernatant. The mixture was then incubated for 10 min at 30 °C and the reaction was terminated by addition of 2 ml of 0.4 M trichloroacetic (TCA) acid. The mixture containing the culture supernatant was then incubated for 30 min at room temperature

followed by centrifugation at 11,000 x g for 5 min. The resultant supernatant (1 ml) was then mixed with 5.0 ml of 0.4 M  $\text{Na}_2\text{CO}_3$  and after 10 min, 1 ml of Folin reagent mixed with 3 ml of distilled water (1:3 v/v) was added to each tube. The tubes were allowed to stand for 30 min at 30 °C and then the absorbance was measured at 660 nm. This similar method was used to prepare the control. However, casein was added only after the reaction was stopped. A standard curve was generated using 0–60 µg/ml of tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate one microgram (1 µg/ml) of tyrosine under the assay conditions described.

### 2.5 Protein estimation

Protein was determined by the method of Lowry [13] using bovine serum albumin (BSA) as the standard.

### 2.6 Purification of enzyme

All purification steps were carried out at 4 °C.

#### 2.6.1 Ammonium sulfate precipitation

Protein precipitation by salting out technique was carried out using ammonium sulfate fractionation with constant and gentle stirring. Two ammonium sulfate precipitation steps were performed in the preparation of enzyme concentrate. The crude enzyme was first saturated up to 30 % (with 17.9 g ammonium sulfate) and then centrifuged at 11,000 x g at 4 °C for 10 min. The supernatant was separated from the pellet, then, the pellet was re-suspended in minimal volume of 0.1M phosphate buffer (pH 7). Approximately 80 % saturation of the separated supernatant was achieved by a slow addition of 52.5 g of ammonium sulfate based on the volume of the supernatant and stirred at cooled condition for an hour. The solution was subsequently transferred into a centrifuge tube and centrifuged at 11,000 x g for 5 min. The final supernatant was discarded and the pellet or precipitate was collected and pooled with the first pellet portion earlier obtained and resuspended in minimal volume of 0.1 M phosphate buffer; pH 7 [14]. The extracts were tested for protease activity and protein content.

#### 2.6.2 Dialysis

A cellulose acetate dialysis tube was treated to remove protectants such as sulfur and glycerin compounds present in it. The treatment of the tube was achieved by boiling the tube in 2 % sodium bicarbonate for 10 minutes to remove sulphides. It was rinsed once in distilled water and

again boiled in 10 mM EDTA solution for 10–20 minutes to remove metals. The tube was cooled and washed extensively in distilled water and stored at 4 °C. One end of the dialysis tube was closed using leak proof clamps. The enzyme product from the ammonium sulphate precipitation were introduced into a cellulose-based dialysis tubing. The other end of the dialysis tube was clamped while keeping sufficient space above the sample and placed in a beaker. The solution was dialyzed against 500 ml of the same buffer for 12 hours at 4 °C in a refrigerator to remove the excess salt with one change after 4 h. This was followed by centrifugation of the resulting solution at 12,500 x g at 4 °C and the supernatant was tested for protease activity and protein concentration [15].

### 2.6.3 Gel filtration

Sephadex G-200 gel was used to further purify the protease enzyme. Preparation of the gel column and the fractionation procedure was as described by Souza et al. [16]. The gel was equilibrated with 0.1 M phosphate buffer of pH 7.0 and the slurry was allowed to swell overnight at room temperature. Sodium azide (0.02 %) was added to prevent microbial growth. The gel was packed into a column (2.6 x 40 cm) and equilibrated with 0.1 M phosphate buffer of pH 7.0. The enzyme solution was collected and dissolved in phosphate buffer 0.1 M, pH 7.0 and fractionated through the Sephadex G – 200 column. Seven milliliters of the enzyme preparation was applied carefully to the top of the gel and allowed to pass into the gel by running the column. Buffer was added without disturbing the gel surface and the reservoir. Elution was carried out with the respective buffer at a flow rate of 20 ml/h. Twenty fractions (5 ml each) were collected and each fraction was analyzed for protein concentration and protease activity. The eluted enzymatically active fractions were pooled and used as the partially purified enzyme [17].

### 2.6.4 Alpha-casein agarose affinity chromatography

For further purification of the sepharose gel filtered sample, it was subjected to purification by alpha-casein agarose gel column chromatography. Preparation of the column and the fractionation procedure was as described by Kocabiyik and Ozdemir [18]. Phosphate buffer (pH 7.0) was used. Five grams of  $\alpha$ -casein agarose was suspended in 0.1 M phosphate buffer and kept overnight for equilibration. The column (2.6 x 40 cm) was carefully packed and equilibrated with the buffer. Three milliliters of the partially purified enzyme extract was diluted to 15 ml

and loaded onto the column and was washed with approximately 100 ml of the equilibration buffer. Proteins was eluted with phosphate buffer pH 7.0 and NaCl gradient 0.1–1 M, was passed through the exchanger at a flow rate of 30 ml/h. Five milliliters fractions were collected. The eluted fractions were collected and each fraction was analyzed for protein concentration and protease activity and the active fractions were pooled together.

### 2.6.5 Determination of molecular weight

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the method suggested by Laemmli [19]. Electrophoresis was performed in a mini- slab gel using 5 % stacking gel and 10 % separating gel. Sample was mixed with sample buffer containing SDS and  $\beta$ -mercaptoethanol and heated at 100 °C for 5 min in water bath before electrophoresis. Electrophoresis was performed at 50 V for stacking gel and 100 V for separating gel. Protein bands was visualized after staining the gel with 0.25 % Coomassie Brilliant Blue R250 in 30 % ethanol- 10 % acetic acid for 20 min and destained with 30 % ethanol-10 % acetic acid. The molecular weight of protein bands was determined by comparing with the bands of standard molecular mass markers [20].

## 2.7 Characterization of purified enzyme

### 2.7.1 Effect of pH on activity and stability of protease

The effect of pH on the enzyme activity of protease was determined with casein (1 % w/v) as substrate by measuring the enzyme activity at varying pH values ranging from 4 to 10 at 40 °C for 10 min using different buffer systems: 0.1M sodium acetate buffer (pH 4.0–5.0); 0.1 M sodium phosphate buffer (6.0–7.0); tris - HCl buffer (pH 8.0) and a glycine – NaOH buffer (pH 9.0–10.0). The pH stability studies were performed by pre-incubating the purified enzyme without substrate in pH values ranging from 4.0 - 10.0 at 40 °C for 2 hours and the residual proteolytic activity was determined under standard assay conditions [21].

### 2.7.2 Effect of temperature on activity and stability of protease

The effect of temperature on protease activity was determined by estimating the protease activity at pH 5.0 within a temperature range of 30–60 °C for 2 hours using casein as substrate. The thermal stability of the protease was determined by pre-incubation of crude enzyme preparation (pH 5.0) at different temperatures (30–60 °C) for 30

min without substrate. The residual proteolytic activity was determined under standard assay conditions [21].

### 2.7.3 Effect of metal ions on enzyme activity

The effects of various metal ions (5 mM) on enzyme activity was determined by pre-incubating the enzyme preparation for 30 minutes at 25 °C in different ionic solutions:  $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$  ( $\text{Ca}^{2+}$ ),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( $\text{Mg}^{2+}$ ),  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  ( $\text{Mn}^{2+}$ ),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ( $\text{Fe}^{3+}$ ),  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$  ( $\text{Cu}^{2+}$ ) and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  ( $\text{Zn}^{2+}$ ). Thereafter, protease assay was performed at 40 °C and pH 5.0. Relative activity was determined by considering the activity of the enzyme without any metallic ions as 100 %. All experiments were conducted in triplicates and their mean values presented [21].

### 2.7.4 Effect of inhibitors on enzyme activity

Protease type was determined by employing the following inhibitors: ethylenediaminetetraacetic acid (EDTA) and phenylsulfonyl fluoride (PMSF). Protease was pre-incubated with each inhibitor, 5.0 mM, in 100mM acetate buffer (pH 5.0) for 30 min at 40 °C. The control was pre-incubated without any inhibitor and the residual activity was calculated [22].

### 2.7.5 Effect of surfactants on enzyme stability

The effect of surfactants: 1 % sodium dodecyl sulphate (SDS), Tween-20 (Polyoxy ethylene sorbitanmonooleate), Tween-80 (polyethylene glycol sorbitanmonooleate) and Triton X-100 (trioctylphenoxypolyethoxyethanol) on the proteolytic activity was determined by pre-incubating enzyme for 1 h in the above surfactants at 40 °C and pH 5.0 before assaying for the residual enzyme activity. A control was kept with the enzyme and the substrate without detergents and the value of the control activity was considered as 100 % [23].

## 2.8 Substrate specificity of protease

Protease activity with various protein substrates including bovine serum albumin (BSA), casein, egg albumin and gelatin was assayed by mixing 1 ml of enzyme and 2 ml of assay buffer (acetate buffer, 0.5 M, pH 5) containing protein substrate (2 mg/ml). After incubation at 40 °C for 30 minute with each of the substrates separately, enzyme activity was measured [24].

## 2.9 Application of protease

The de-staining property was studied by applying human blood on four pieces of clean white cloth (5 cm x 7 cm) and

allowed to air dry for 15 min. The stained cloth pieces were taken into four different flasks as described by Kunamneni et al. [25]. The first flask was prepared with 100 ml of distilled water, stained cloth and 1ml of detergent (7 mg/ml). The second was prepared with 100 ml of distilled water, stained cloth and 1 ml of enzyme extract. The third was prepared with 100 ml of water, stained cloth, 1 ml of detergent (7 mg/ml) and 1 ml of enzyme extract. The last flask was prepared with 100 ml of distilled water and stained cloth as negative control. The flasks were incubated at 40 °C for 15 minutes. After incubation, cloth pieces were taken out, rinsed with water, and dried. Visual examination of various pieces showed the effect of enzyme in the removal of stains.

## 3 Results and discussion

### 3.1 Purification of protease

A 7.7 – fold purification was achieved with 20 % recovery of protease activity, yielding a specific activity of 93.88 U/mg protein. The result obtained in this research as shown in Table 1 present an 11 % yield. This value was higher than the yield reported for the fibrinolytic enzyme from a *Fusarium culmorum* [26]. The purified enzyme preparation appeared as a single protein with a molecular weight of 70 kDa (Fig. 1). In variance to our findings, the molecular weight of the *F. oxysporum* FWT1 protease was higher than that reported by Ricardo et al. [27], Pekkarinen et al. [26] and Wu et al. [28] having a molecular mass of 41, 28.7 and 28 kDa for purified protease by *Fusarium culmorum*, *Fusarium oxysporum* var *iini* and *Fusarium* sp. respectively. Griffen et al. [29] also reported that aspartic protease by *F. graminearum* had molecular mass of 40 kDa. With respect to results obtained, a similar protein size of 70 kDa on SDS-PAGE was reported by Na and Liang [30].

**Table 1** Purification steps of protease produced by *Fusarium oxysporum* FWT1

Purification steps	Enzyme activity (U/ml)	Total protein (mg)	Specific activity (U/mg)	Yield %	Purification fold
Crude enzyme extract	1826±2.0	150±2.5	12.17±1.62	100	1.0
Ammonium precipitation	486±1.0	21±1.0	23.14±1.78	27	1.9
Dialysis	482±2.0	17±1.5	29.35±2.5	26	2.4
Sephadex G-200	432±0.75	6.5±0.25	61.71±2.0	24	5.1
$\alpha$ -casein agarose	375±0.5	4.0±0.3	93.88±2.71	20	7.7

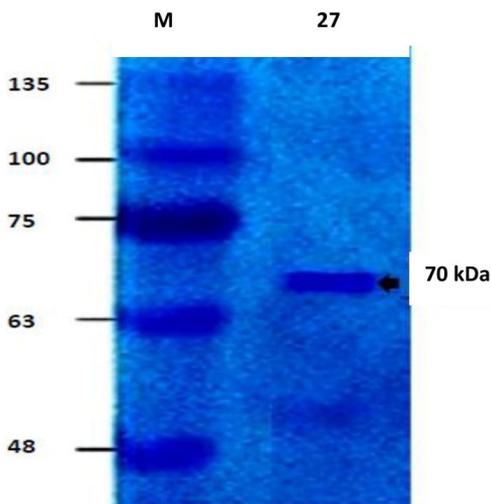


Fig. 1 SDS PAGE of purified protease. M: molecular mass maker, Lane 27: purified protease of *Fusarium oxysporum* FWT1.

### 3.2 Characterization of the purified enzyme

#### 3.2.1 Effect of pH on enzyme activity and stability of protease

The optimum pH of purified protease was at pH 5 (100 %). Protease activity decreased as the pH increased from pH 6–9 with the least activity at pH 10 (Fig. 2). Fungal proteases are reported to be active over a wide pH range (pH 4.0 to 11.0) [31], the enzyme produced however appeared to be slightly acid. Fungi have been characterized as good producers of acid proteases [32], with the enzymes showing optimal pH between 4.0 and 5.0 [33]. Adejuwon and Olutiola [34] in their work reported optimum pH for protease activity at 6 by *Fusarium oxysporum*. pH stability at wide range of pH is a desirable property of any enzyme for industrial application. Pekkarinen et al. [26] reported that the enzyme was active at pH 8.3–9.6 by *F. culmorum*. A pH of 5 has been reported to be the optimum pH for other fungal protease such as those of *Aspergillus* sp. and *Chaetomium thermophilum* [24, 35].

#### 3.2.2 Effect of temperature on enzyme activity and stability of protease

The result shown in Fig. 3 indicates 40 °C (100 %) as the optimum temperature of activity for the purified protease. Further increase in temperature was detrimental to enzyme activity with the least activity at 60 °C. Reports by Adejuwon and Olutiola [34] had corroborated our observations as temperature values of 35–40 °C had been shown to be optimal for *Fusarium* spp. protease produced. The enzyme displayed good stability over temperatures ranging from 30–40 °C; the optimum temperature was found to

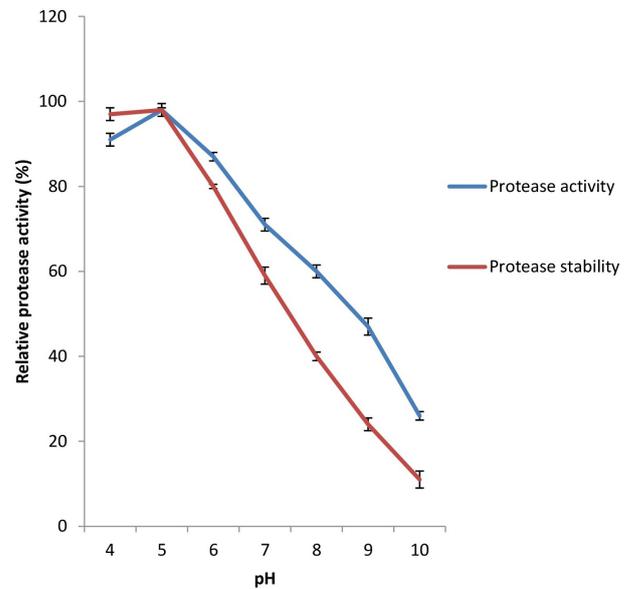


Fig. 2 Effect of pH on the activity and stability of purified protease from *Fusarium oxysporum* FWT1

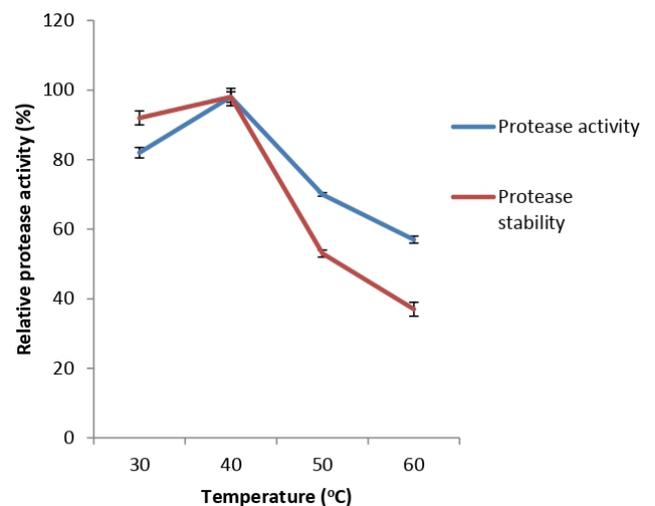


Fig. 3 Effect of temperature on the activity and stability of purified protease from *Fusarium oxysporum* FWT1

be 40 °C while at 30 °C it showed 92 % proteolytic activity. The residual activity of the enzyme showed a sharp decrease in proteolytic activity between 40 and 60 °C (37 %) as the temperature increased. Pekkarinen et al. [26] also reported enzyme stability at 50 °C by *Fusarium culmorum*, a value higher than as observed in our experiments. Variations in temperature stabilities could be strain specific and specific enzyme properties molecular characteristics could reveal further these variation. Proteases from other fungi like *Aspergillus carneus*, *Penicillium janthinellum* and *Aspergillus tamarii* showed optimum stability at 40 °C [14, 36–38] but unstable at temperatures above 40 °C. Overall, the temperature optima and stability profile

of the purified protease suggested that the enzyme could be a viable option for industrial application.

### 3.2.3 Effect of metal ions on the activity of protease

The effect of various metal ions on enzyme activity result is presented in Table 2. It showed that under the metal ion  $Mg^{2+}$  the enzyme was stable with no noticeable reduction in its activity. The effect of  $Cu^{2+}$  which elicited a 28 % relative protease activity compared with the control showed there was a drastic reduction in enzyme activity as  $Cu^{2+}$  was the most potent inhibitor of protease. The result obtained in this research was in conformity with the earlier findings of Adejuwon and Olutiola [34] which showed enhancement of protease activity in the presence of  $Ca^{2+}$  and  $Mg^{2+}$  by *Fusarium* spp. Reports by Namrata and Kantishree [39] and Ricardo et al. [27] for *Aspergillus tamaris* and *Fusarium oxysporum* were also in conformity with our data. This is because the metal ions had been stated as viable stability enhancers of proteases. The ions  $Ca^{2+}$  and  $Mg^{2+}$  are involved in stabilizing the active structure, but they are not needed for the catalytic function or for the protein chain folding process to form the active structure [40, 41]. Based on metal ion interaction with other enzymes, it had also been identified that  $Cu^{2+}$  and  $Co^{+2}$  had inhibitory effects on metallo fibrinolytic enzymes of *Fusarium* spp. [28] having a direct correlation with our results for  $Cu^{2+}$ .

### 3.2.4 Effect of inhibitors and surfactants on the activity of protease

The effect of inhibitors and surfactants on enzyme activity studied is as shown in Table 3. It was noted that Tween-20 and Tween-80 (100 and 95 %) had no effect on protease activity while inhibition of enzymatic activity was most noticed for EDTA and PMSF (26 and 36 % of residual activity respectively). Inhibitory effects of PMSF were also reported by Pekkarinen et al. [26], Namrata, and

**Table 2** Effect of Metal ion on the activity of purified protease from *Fusarium oxysporum* FWT1

Metal ion Source	Relative protease activity (%)
Control	100
$MnSO_4$ ( $Mn^{2+}$ )	52
$FeSO_4$ ( $Fe^{2+}$ )	41
$CuSO_4$ ( $Cu^{2+}$ )	28
$ZnSO_4$ ( $Zn^{2+}$ )	51
$MgSO_4$ ( $Mg^{2+}$ )	100
$CaCl_2$ ( $Ca^{2+}$ )	84

Kantishree [39] and Sharma and Kantishree [22] using *Fusarium culmorum*, *Rhizopus microsporus* NRRL3671 and *Aspergillus tamaris* [EF661565.1]. They also further concretized their findings by stating that their inhibition by PMSF suggested that these enzymes were serine proteases. It is an observable fact that surfactants alter cell permeability of microorganisms thereby leading to increased protein secretion or surface effects on cell-bound enzymes [42]. Due to their specificity, inhibitors and surfactants can also aid in characterization of novel proteases by examining hydrolysis rates of protease in their presence [17].

### 3.2.5 Effect of different substrates on the activity of protease

Protease activity measured under the influence of various substrates including bovine serum albumin, casein, gelatin and egg albumin is as shown in Table 4. Casein (100 %) was found to be the best substrate for protease activity with the least proteolytic activity observed by bovine serum albumin (49 %). Similar reports of a preference for caseinolysis over other substrates had been stated for *Aspergillus niger* [17]. The purified protease could hydrolyze all substrates tested but showed higher specificity to casein hydrolysis than the other three substrates. The important feature of proteases is their ability to discriminate among competing substrates and utility of these enzymes often depends on their substrate specificity [43, 44]. The substrate specificity profile of the purified protease indicated that the enzyme had a broad range of hydrolytic activity on various protein substrates which shows a great potential in biotechnological applications.

**Table 3** Effect of surfactants and inhibitors on protease activity

Surfactants and Inhibitors	Relative protease activity (%)
SDS	66
Tween 20	100
Tween 80	95
Triton X-100	91
EDTA	26
PMSF	36
Control	100

**Table 4** Effect of different substrates on the activity of protease

Substrate	Relative protease activity (%)
Casein	100
Bovine serum albumin	49
Egg albumin	87
Gelatin	69

### 3.3 Application of protease in de-staining (Washing tests)

The de-staining studies gave good results on the cotton cloths stained with human blood (Fig. 4). There was still an evident blood stain in the cloth washed with only water (A). However, de-staining effects were observed for the other cloth samples treated with detergent solution alone (C), detergent mixed with enzyme (B), and enzyme alone (D). The enzyme used was the purified form ( $1 \text{ ml}; 375 \pm 0.05 \text{ U/ml}$ ), and these results showed the effectiveness of the extracted protease in proteinaceous stain removal. This indicates that the protease enzyme obtained from this isolate could be considered as a potential candidate for use as cleaning agent to facilitate the release of proteinaceous stains and also of great significance in textile industries. Studies on the usefulness of proteases from fungi were reported [45] in the facilitation of blood stains removal from cotton cloth both in the presence and absence of detergents.

### 4 Conclusion

In this present study, protease produced by *Fusarium oxysporum* was purified to 7.7 fold with a yield of 20 %. The molecular weight of the purified enzyme was determined to be approximately 70 kDa. The enzyme was optimally active and stable at pH of 5.0 and at 40 °C. This study also highlighted the possibility of co-application of the produced protease with detergent for effective stain removal. Though there were no valid compatibility

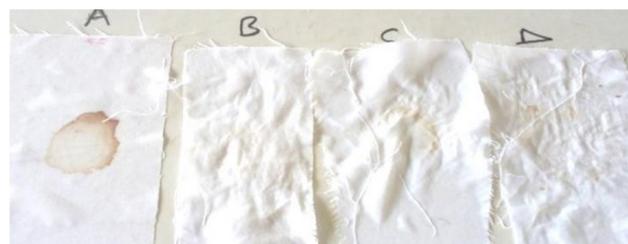


Fig. 4 Blood stain removal using fungal protease and Detergent

assessments carried out with respect to the efficaciousness of the enzyme in combination with the detergent. The de-staining experiments have showed that the enzyme has hydrolytic effects on stains with or without the addition of detergent. Further research is however needed to determine the compatibility levels between the enzyme and detergents of various types based on biochemical and chemical properties, as well as design a variety of effective methods for the application of this enzyme. *Fusarium oxysporum* FWT1 therefore is a potentially good source of stable enzyme for industrial applications.

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