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Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials

Raphael Emuebie Okonji[1], Babamotemi Oluwasola Itakorode[2*], Julius Ovie Ovumedia[1] and Odunayo Selimot Adedeji[3]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, [2]Department of Chemical Sciences (Biochemistry Unit), Oduduwa University Ipetumodu, Ile-Ife, Nigeria, [3]Department of Biochemistry, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria

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ABSTRACT

This study isolated, screened, and identified a pectinase-producing fungus from a decomposing plant material. It also cultured the isolated fungus under optimized conditions to obtain crude pectinase enzyme as well as purified and investigated the biochemical characteristics of the purified enzyme. The fungal strain was isolated on pectinase screening agar medium containing 1% pectin and obtained a clear zone. It was identified as *Aspergillus fumigatus* and cultivated for enzyme production using banana, plantain, and orange peels as the solid substrate. Under optimized conditions, a maximum of 3.52 U/ml pectinase activity was obtained at 65% moisture content after 144 h (6 days) of incubation period on orange peel, 1.5 ml inoculum, and 3% salt content. *A. fumigatus* pectinase was purified 4.45-fold and a yield of 26.16% with a specific activity of 38.88 U/mg. The molecular weight determined on sodium dodecyl sulfate (SDS-PAGE) was 31.6 kDa. The pectinase exhibited maximum activity at 60°C, optimum pH of 5.0, and stability at 40–50°C. The enzyme showed a preference for polygalacturonic acid as its primary substrate with a K_M 3.08 mg/ml and V_{max} of 1.61 U/ml. The enzyme was activated by 0.5 mM Na^+, K^+, and 1–5% toluene. The enzyme activity was inhibited by metal cations; 20% ethanol, 4.0 mM SDS, and L-cysteine. The obtained results showed that *A. fumigatus* pectinase could be a candidate for potential industrial and biotechnological application.

1. INTRODUCTION

The use of enzyme technology to meet various human needs is becoming popular. Enzymes are used extensively in industry to improve the production of fruit juices, fruits texture, and enzymatic peeling of fruits [1-3]. It has been used in the textile industry as well as coffee and tea fermentation [4,5]. Pectinases are enzymes that are responsible for the biological degradation of pectin, a large molecular weight polysaccharide found in plants [6,7]. One of the most important processes in biomass degradation is the biological decomposition of pectin [8,9]. In plants, pectinases play a diverse role such as cell-to-cell adhesion, a source of signaling molecules, and in the ripening of fruits [10]. Pectinases are also important in plant pathogenesis and have been reported to be the first enzyme synthesized by certain fungal and bacterial pathogens grown on isolated plant cell walls so as to "prepare" the cell wall components for subsequent degradation by other enzymes [11]. Pectinases are also important industrial enzymes used in the production of fruit juices and wines [5,12]. Vegetable oil extraction can be augmented by applying enzymes such as pectinase [5]. Pectinases are secreted by a wide range of microorganisms such as bacteria and fungi [13,14] and it accounts for 25% of the global food enzyme sales [5]. The microorganisms that are used for the production of commercially important enzymes such as pectinases must be "generally regarded as safe" and able to secrete the enzyme in large quantities to the extracellular environment for easy extraction of the enzyme [15]. The preference for a microbial source is usually due to the low cost of production, enzyme content is more predictable and controllable, availability of raw materials for production, and mass production without ethical clearance [16]. The cost of production can be reduced by optimizing the enzyme production conditions [17]. The high cost of the production, as well as the stability of pectinases in an industrial process, is conceivably the major constraint in the commercialization of the new sources of pectinase. Given the biotechnological potential of pectinolytic enzymes, microbial production of pectinases has been extensively studied in recent years [5,17-19]. Thus, the study of physicochemical properties of pectinase produced by *Aspergillus fumigatus* may help in the development of products which will intervene in plants diseases caused by pectinase-secreting pathogen organisms and also help in eliminating the use of carcinogenic organic solvents in industrial processes.

**Corresponding Author:*
Babamotemi Oluwasola Itakorode, Department of Chemical Sciences, Biochemistry Unit, Oduduwa University Ipetumodu, Ile-Ife, Nigeria.
E-mail: itakorgsoli@yahoo.com

2. MATERIALS AND METHODS

2.1. Collection of Samples

Agro wastes such as orange peels, banana peels, and plantain peels were collected from local market and dumpsite in Omole Estate, Ile-Ife. The wastes were oven dried for 36 h at 80°C, pulverized, and stored in an airtight container. The pulverized samples were mixed with soil and left to decay in a container for several weeks. Soil samples around the decomposing peels were collected in sterile bottles and taken to the laboratory for analysis.

2.2. Isolation of Fungi and Screening for Pectinase Production

About 1 g of the collected soil sample was serially diluted and 1 ml of the diluted sample was taken into sterilized Petri plates. Agar solution was then poured into the plates using the pour plate method, allowed to set, and incubated at 37°C for 72 h. The cultures were subsequently subcultured until pure isolates were obtained. The screening for pectinase production was done using pectin agar plate [20]. Briefly, pure cultures were inoculated into sterilized and solidified pectin agar medium. Inoculated plates were incubated at 37°C for 48 h. Thereafter, the plates were flooded with iodine-potassium iodide solutions for 5–10 min and pectinase-producing colonies were detected by the appearance of a clear zone around them. The fungi isolate with the highest value of the clear zone of the hydrolysis of pectinase was selected and stored in the fridge on pectin agar.

2.3. Identification of the Pectinase-producing Isolate

The selected fungus with the highest value of pectinase hydrolysis was macromorphologically characterized by observing colony characteristics such as color, texture, and spore structure according to the handbook for the identification of fungi [21] and micromorphologically by employing conventional lactophenol cotton blue technique (LPCB).

2.4. Pectinase Production using Solid State Fermentation

About 10 g of solid substrates, consisting of 99.7% (9.97 g) each of the powdered plant peels, 0.1% (0.01 g) $(NH4)_2SO_4$, 0.1% $MgSO_4$, and 0.1% K_2HPO_4, were weighed into 250 ml Erlenmeyer's flasks and then autoclaved at 121°C for 20 min. After cooling, 1 ml of the standard inoculum was introduced and the final moisture content adjusted to 70% using distilled water. The flasks were incubated for 14 days at 37°C. Following fermentation, 60 ml of citrate buffer (0.1 M, pH 5.0) was added to the flasks and stirred for 30 min under ice. The mixture was filtered and centrifuged at 12,000 rpm for 20 min. The resulting supernatant was tested for pectinase activity.

2.5. Pectinase Assay Method

The enzyme activity was determined using pectin as a substrate. The assay mixture consists of 0.80 ml of the substrate solution (1.0% w/v citric pectin in 0.1 M citric buffer pH 5.0) and 0.20 ml of enzyme solution. The reaction medium was incubated at 50°C for 20 min and terminated by the addition of 1.5 ml of 3, 5-dinitrosalicylic acid reagent [22]. The absorbance of the reaction medium was taken at 540 nm. One unit of pectinase activity (U) was defined as 1 µmol reducing sugar released per minute using galacturonic acid as standard.

2.6. Protein Concentration Determination

The protein concentration was estimated by the method of Bradford [23]. Bovine serum albumin (BSA) was used as standard.

2.7. Optimization of Pectinase Production

The step-wise optimization of pectinase produced by the isolate was studied for four optimization parameters: Incubation time, moisture content, the volume of inoculum, and $(NH_4)_2SO_4$ salt concentration.

2.7.1. Effect of incubation time, ammonium salt concentration, and moisture content on pectinase production

Production medium was prepared into seven 250 ml flasks, at intervals of 48 h up to 14 days, a flask was taken and the crude enzyme extracted for enzyme activity and protein concentration [24]. Powdered orange peel was used as the solid substrate. The moisture content was adjusted from 40 to 80% at 5% interval while the inoculum volume was varied from 0.5 ml to 4.0 ml. After 6 days, the crude enzyme was extracted and checked for enzyme activity. The effect of ammonium salt concentration was checked by varying $(NH_4)_2SO_4$ salt from 0.1% to 4.0%.

2.8. Purification Procedure

2.8.1. Pectinase purification on CM-Sephadex C-50 and Sephacryl S-200

Crude pectinase produced was subjected to 80% ammonium sulfate saturation and left overnight in the refrigerator. The resulting precipitate was dissolved in a small volume of 0.1 M citrate buffer (pH 5.0) and dialyzed extensively in cold 0.1 M citrate buffer (pH 5.0). 5 ml of the concentrated enzyme was layered on CM-Sephadex C-50 column equilibrated with 0.1 M citrate buffer (pH 5.0). Fractions were collected at a rate of 15 ml/h. Unbound protein was removed by citrate buffer (0.1 M, pH 5.0) followed by a step-wise elution with 0.5 M and 1.0 M NaC1 in the buffer. Pectinase activity and protein concentration of the fractions were determined. The active fractions were pooled and dialyzed against 50% glycerol in 0.1 M citrate buffer, pH 5.0. 5 ml of the concentrated enzyme was layered on a Sephacryl S-200 column. The column was eluted with 0.1 M citrate buffer, pH 5.0 at the flow rate of 20 ml/h. Pectinase activity was checked while protein concentration was monitored spectrophotometrically at 280 nm.

2.8.2. Native molecular weight determination

The native molecular weight was determined on Sephacryl S-200 column (1.5 cm × 65.5 cm). The standard proteins were lysozyme (14,000 Da; 15 mg/ml), trypsin (24,000 Da; 10 mg/ml), pepsin (35,000 Da; 10 mg/ml), ovalbumin (45,000 Da; 10 mg/ml), and BSA (66,000; 10 mg/ml). Fractions of 5.0 ml were collected and monitored spectrophotometrically and the elution volume of each protein was estimated. Void volume (V_0) was determined using blue dextran (2 mg/ml). The pure enzyme (5.0 ml) was then passed through the same column and the elution volume of the pectinase was estimated. The method of Laemmli [25] was used to determine the subunit molecular weight.

2.9. Characterization of the Enzyme

2.9.1. Kinetic parameter determination

The kinetic parameters (V_{max} and K_M) of the enzyme were determined for three substrates: 67% methoxylated citric pectin, 7.8% methoxylated apple pectin, and polygalacturonic acid (PGA). The concentrations were varied from 1 to 8 mg/ml and the initial reaction velocities were determined. The data were plotted according to the method of Lineweaver and Burk [26].

2.9.2. Effect of Temperature and pH

The optimum temperature of the enzyme was assayed using the method of Tari *et al.* at temperatures between 30°C and 90°C. Citric pectin was

used as the substrate. Effect of pH was studied using citrate buffer (4.0–5.0), phosphate buffer (6.0–8.0), and borate buffer (9.0–10.0). Citric pectin was used as substrate.

2.9.3. Thermal Stability of A. fumigatus Pectinase Activity

The enzyme was pre-incubated in a 0.1 M citrate buffer within a temperature range of 40–70°C. For a time span of 120 min at intervals of 15 min, aliquots of enzyme samples were withdrawn and the residual activity was determined.

2.9.4. Effect of Salts on A. fumigatus Pectinase Activity

Influence of various salts at a different concentration on the enzyme activity was checked. The tested salts were NaCl, KCl, NH_4Cl, $SnCl_2$, $CaCl_2$, $BaCl_2$, $MnCl_2$, $MgCl_2$, and $AlCl_3$. The enzyme was pre-incubated for 10 min at 60°C with the various salts, before the addition of pectin; then, the residual pectinase activity was evaluated. Assay mixture without salts was taken as control.

2.9.5. Effect of organic solvents on A. fumigatus pectinase activity

Pectinase assay was performed in the presence of 1%, 5%, 10%, and 20% of various organic solvents. The organic solvents used include hexane, methanol, ethanol, acetone, toluene, and diethyl ether (DEE). The enzyme activity was checked as previously described.

2.9.6. Inhibition of pectinase activity

The effects of known enzyme inhibitors such as ethylenediaminetetraacetic acid (EDTA), beta-mercaptoethanol (BME), Triton X-100, sodium dodecyl sulfate (SDS) and Tween-80, and L-cysteine on the pectinase activity were studied. The enzyme was pre-incubated with 0.5 mM and 1.0 mM, 2.0 mM, 3.0 mM, and 4.0 mM of these inhibitors before the addition of substrate. Reactions in the absence of these inhibitors were used as the control with 100% enzyme activity. All chemicals were solubilized in distilled water.

3. RESULTS AND DISCUSSION

3.1. Isolation, Screening, and Optimization of *A. fumigatus*

This study made an attempt to isolate the fungus capable of producing pectinase, from the soil of decomposing plant materials. Among all the isolates, *A. fumigatus* showed the maximum pectinase activity based on carbohydrase hydrolysis of pectin in pectin-agar plate assay. *A. fumigatus* was identified based on microscopic and macroscopic examinations on LPCB. *A. fumigatus* is a saprophytic fungus that is found widespread in nature. This organism has been found to reproduce not only asexually but also has a functional cycle of sexual reproductive [27]. Furthermore, the fungus can grow at a temperature range of 37–50°C [28].

The clear zone of hydrolysis around the selected strain is shown in Figure 1. When subjected to pectinase production, orange peel produced the highest pectinase activity as well as total protein concentration (Figure 2). Maximum pectinase activity (1.75 U/ml) was observed at 6 days of incubation with a steady decline of activity after 6 days (Figure 3a). The best moisture content for *A. fumigatus* (65%) produced a pectinase activity of 2.3 U/ml (Figure 3b). Furthermore, highest pectinase production was also observed at 1.5 ml inoculum volume (Figure 3c) and 3% salt concentration (Figure 3d), respectively. Similar observations on the orange peel as an excellent carbon source for pectinase production have been reported [29,30]. The reason for the highest pectinase activity using orange peel as solid substrate could be the high percentage of pectin found in the cell wall of the peel. Several

Figure 1: Screening of fungi for pectinase activity

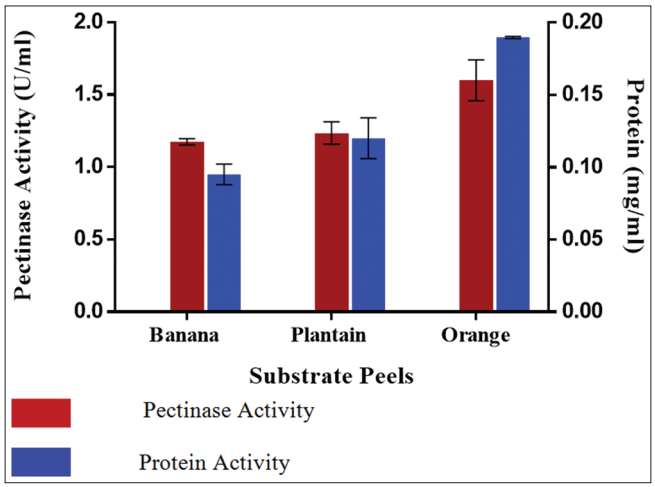

Figure 2: Pectinase activities and protein concentrations using different solid substrate

works have been carried out to show that pectinolytic fungi produce pectinase maximally between 3 and 6 days incubation period. The ideal substrate humidity for pectinase production by *Aspergillus foetidus* was reported to be between 80% and 90%.

3.2. Purification of *A. fumigatus* Pectinase

A. fumigatus pectinase was purified with a fold of 4.45, a yield of 26.14%, and specific activity of 38.88 U/mg of protein (Table 1). The elution profiles after CM Sephadex C-50 ion-exchange chromatography and Sephacryl S-200 are shown in Figure 4 and 5, respectively. The molecular weight of native and denatured pectinase was determined using the marker proteins lysozyme (14.5kDa), trypsin (24 kDa), pepsin (35 kDa), ovalbumin (45kDa), and BSA (66 kDa) to be about 32.5 kDa by gel filtration and SDS PAGE, respectively, indicating that the enzyme is of monomer in nature (Figure 6). Microbial pectinases have been reported to be monomeric in structure and generally fall within the size range of 30–70 kDa [31-35]. Two endopolygalacturonases PGI and PGII purified from *A. japonicas* showed the molecular weight of 38 and 65 kDa, respectively [36]. Polygalacturonase isolated from *A. awomori* showed a molecular weight of 41 kDa [37]. Furthermore, pectinase isolated from *Bacillus licheniformis* showed a molecular weight of 38 kDa [38].

Table 1: Summary of the purification process for *Aspergillus fumigatus* pectinase

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude sample	21.63	188.74	8.73	100.00	1.00
0–80% $(NH_4)_2SO_4$ precipitation	12.29	128.53	10.66	68.09	1.22
Ion-exchange chromatography	3.40	51.64	15.19	27.36	1.74
Sephacryl-200 gel filtration	1.21	49.38	38.88	26.16	4.45

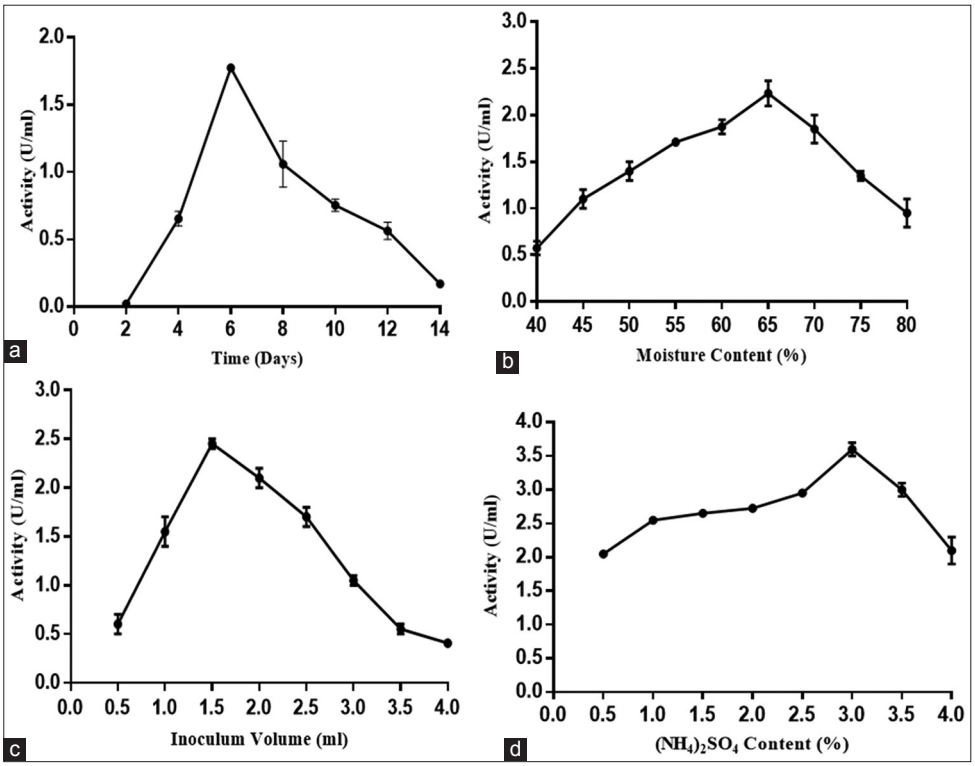

Figure 3: Effect of optimization parameters on *Aspergillus fumigatus* pectinase production. (a) Effect of incubation time on the pectinase production, (b) effect of moisture content on the pectinase production at 6 days of the incubation, (c) effect of inoculum volume on pectinase production at 6 days of incubation period and 65% moisture content, (d) effect of ammonium sulfate concentration at 6 days incubation period, 65% moisture content, and 1.5 ml inoculum.

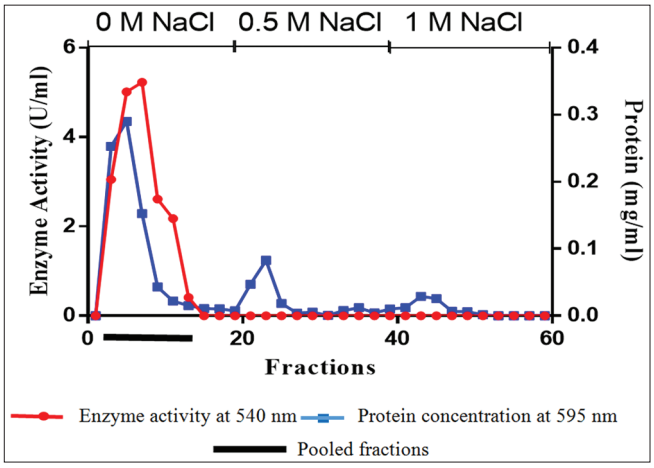

Figure 4: CM-Sephadex C-50 ion-exchange chromatography.

3.3. Kinetic Parameters of *A. fumigatus* Pectinase

The kinetic parameters (K_m and Vmax) of *A. fumigatus* pectinase for citrus pectin, apple pectin, and PGA as substrate are 7.11, 6.01, and 3.08 mg/ml, respectively. Kinetic data are summarized in Table 2. *A. fumigatus* has a higher affinity for PGA. Several K_M values in the range of 1–9 mg/ml have been reported for polygalacturonases [39-48]. Exo-polygalacturonase from *Penicillium frequentans* was shown to have a K_M of 1.6 mg/ml [41]. A value of 0.22 mg/ml was reported for *Rhizomucor pusillus* pectinase using PGA as substrate [42]. It can be said that the kinetic parameters of pectinases vary with the enzyme source and nature substrate used for the assay. In most cases, the K_M value is low, which is in agreement with the result of the present study.

3.4. Effect of pH and Temperature on *A. fumigatus* Pectinase Activity

A. fumigatus pectinase activity was optimum at 60°C as shown in Figure 7. The thermal stability of the pectinase showed that at 60°C, the enzyme retained 100% activity for 45 min and losing about 50% activity at the end of 120 min of incubation (Figure 8). Similar results were obtained for pectinase from other sources [9,43], as well as from *Aspergillus* spp. [5]. These results indicate that for industrial application of the pectinase from *A. fumigatus*, the suitable temperature range could be 40–50°C. *A. fumigatus* pectinase has an optimum pH at 5.0 and decreases significantly below and above this value (Figure 9).

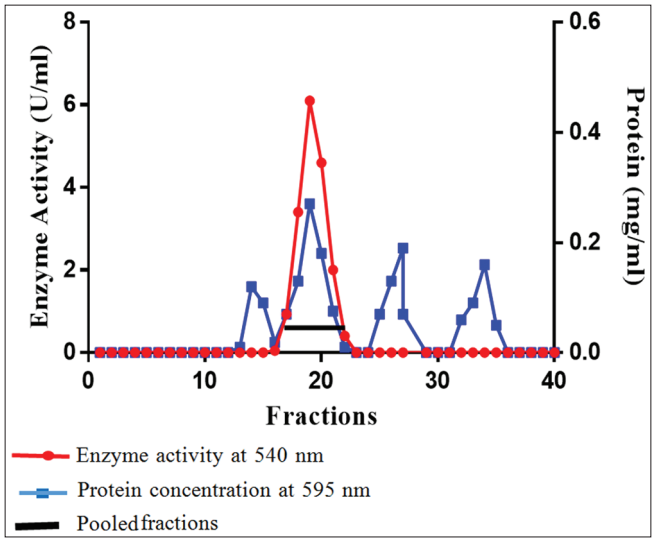

Fig. 5: Sephacryl S-200 gel filtration chromatography.

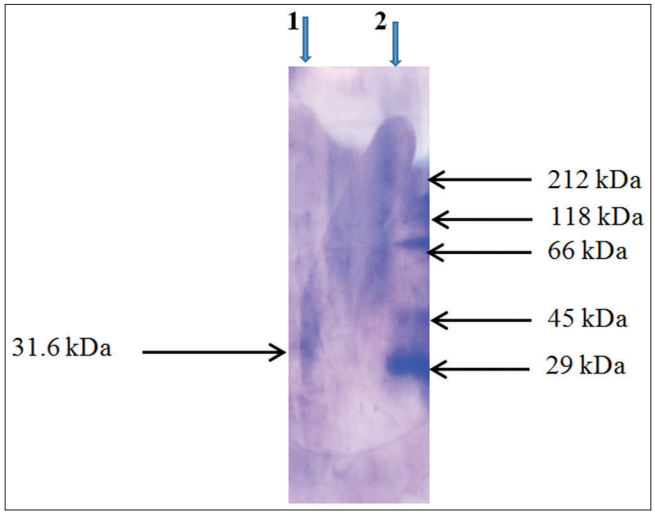

Figure 6: Electropherogram of SDS-PAGE of *Aspergillus fumigatus* pectinase. Lane 1 is *A. fumigatus* pectinase while Lane 2 is the molecular weight ladder

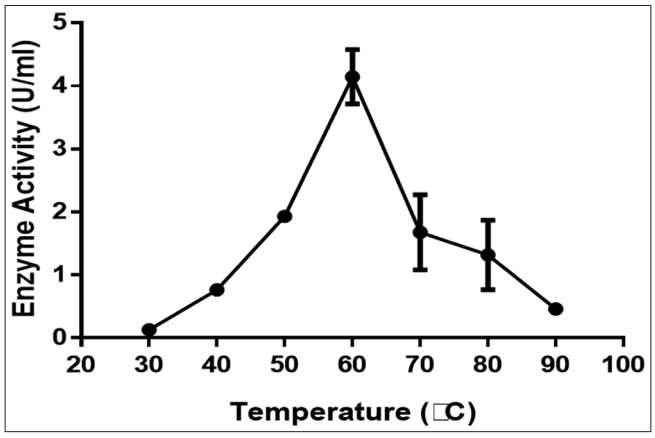

Figure 7: Effect of temperature on the activity of *Aspergillus fumigatus* pectinase

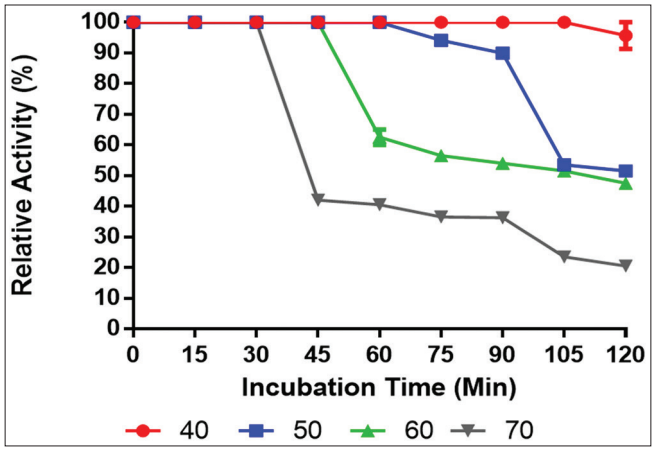

Figure 8: Thermal stability of *Aspergillus fumigatus* pectinase activity

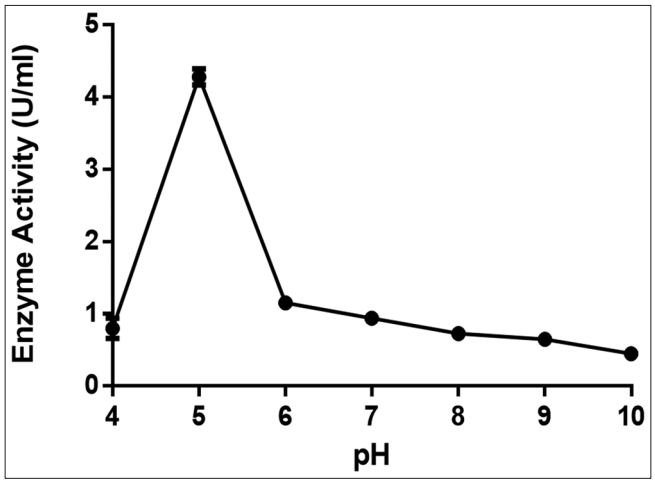

Figure 9: Effect of pH on the pectinase activity of *Aspergillus fumigatus* activity

Similar results have been reported from previous work on *Aspergillus* spp. [5]. Acidic pectinases are produced generally by fungi, especially *Aspergillus spp*. [44]. Therefore, *A. fumigatus* pectinase can be one of the candidates of acid pectinases, which can be employed in the extraction of pectin in fruit juices, in winemaking, and in maceration of vegetables.

The values shown represent the average of duplicate experiments. Error bars represent the standard deviation.

3.5. Effect of Salts, Organic Solvents, and Inhibitors on *A. fumigatus* Pectinase Activity

Table 3 presents the effect of monovalent, bivalent, and trivalent chloride salts on *A. fumigatus* pectinase. The result shows that the pectinase activity increased significantly by the monovalent ions but decreases by divalent ions and the trivalent ion. Activation of the pectinolytic enzyme has been reported [45]. This increase in the enzyme activity may be due to charge neutralization by Na^+, K^+, and NH_4^+ on the pectin polymer [12], so as to reduce the repulsion between the pectin and the overall negative charge of the enzyme. Inhibition by all salt of divalent cations such as $CaCl_2$ and $MgCl_2$ may be due to either the formation of homogalacturonan chain cross-link by the Ca^{2+} and Mg^{2+} cation [46]; thus, decreasing the

availability of the substrate to the enzyme or the binding of the cations to an amino acid side chain involved in the binding or catalysis of the substrate.

The influence of various organic solvents on *A. fumigatus* pectinase activity is shown in Table 4. Hexane, toluene, and DEE with low ε values of 1.9, 2.4, and 4.3, respectively, increase the pectinase activity. This increase in activity is more significant (approximately 35%) with hexane than with other non-polar solvents. The increase in pectinase activity by the non-polar organic solvents may be due to the increase in the concentration of the substrate pectin in the aqueous environment due to the insolubility of pectin in the non-polar organic solvent; thus, making the pectin more available for enzyme action [47]. On the other hand, the polar solvents acetone, ethanol, and methanol with ε values of 21, 25, and 33 respectively, decrease the pectinase activity of *A. fumigatus*. This decrease is more significant with ethanol and methanol at 20% solvent concentration. Therefore, *A. fumigatus* pectinase can be a potential candidate in winemaking where the alcohol percentage is <10% or even between 10% and 15% alcohol concentrations. A similar result on the inhibitory effect of ethanol, which is an important product in the winemaking, has been shown by Merin *et al.* [48].

Table 2: Kinetic parameters of the *Aspergillus fumigatus* purified pectinase

Substrate	K_M (mg/ml)	V_{max} (µmol/min/ml)
PGA	3.08	1.61
Apple pectin	6.10	3.39
Citrus pectin	7.11	4.24

PGA: Polygalacturonic acid

Table 3: The effect of salts on *Aspergillus fumigatus* pectinase activity

Residual activity (%)	0.1 mM	0.5 mM	1 mM
Control	100.00	100.00	100.00
NaCl	178.48±5.21	203.80±10.76	135.87±7.93
KCl	154.01±3.56	195.78±6.17	145.15±2.99
NH_4Cl	162.02±4.99	185.48±9.66	127.51±8.27
$CaCl_2$	96.62±2.44	24.89±1.43	8.44±1.01
$BaCl_2$	91.14±5.87	54.43±7.13	6.33±0.56
$HgCl_2$	94.51±3.11	89.03±5.20	19.83±1.39
$MgCl_2$	92.68±1.65	68.01±1.91	23.33±2.18
$MnCl_2$	89.65±5.52	55.34±3.89	16.01±0.90
$SnCl_2$	98.66±0.91	76.98±2.09	56.11±4.41
$AlCl_3$	89.16±1.92	74.23±5. 60	59.71±3.77

Table 4: Effect of organic solvents on *Aspergillus fumigatus* pectinase activity

Residual activity (%)	1%	5%	10%	20%
Control	100.00	100.00	100.00	100.00
Hexane	134.64±2.13	138.64±3.40	135.20±4.92	136.31±1.35
Toluene	115.08±1.56	115.58±1.23	106.53±0.71	96.98±1.51
DEE	106.98±1.05	105.99±0.75	101. 73±0.23	92.46±0.65
Acetone	95.98±2.70	94.18±1.70	96.36±0.71	93.47±3.26
Ethanol	96.65±1.06	94.41±1.98	90.50±2.92	58.66±5.24
Methanol	91.06±0.65	88.27±1.34	87.71±4.34	35.20±3.13

Influence of various inhibitors on *A. fumigatus* pectinase activity is presented in Table 5. The effect of inhibitors on enzyme activity can provide important information on the primary and secondary structure of the protein. SDS, Tween-80, and L-cysteine affect the pectinase activity significantly at 4.0 mM inhibitor concentration. The effect of BME on the activity was insignificant for all the concentrations studied (0.5–4.0 mM). BME reduces disulfide bonds and can act as a biological antioxidant. This result suggests that there is no vulnerable disulfide bond holding the structure of the pectinase together, meaning that the pectinase from *A. fumigatus* is a monomeric enzyme as already confirmed by the calculations made from the native and subunit molecular weights. The decrease (10–13%) in the pectinase activity by EDTA is constant from 0.5 to 4.0 mM. This suggests that EDTA decreases the pectinase activity by means other than chelating the pectinase enzymes together. Therefore, *A. fumigatus* pectinase is not a metalloenzyme. However, the stimulatory and inhibitory effect of EDTA on *Penicillium oxalicum* polygalacturonase has been reported [49,50]. The effect of EDTA also suggests that *A. fumigatus* pectinase is not a polymethyl-galacturonate lyase, which requires Ca^{2+} and is strongly inhibited by EDTA [5]. SDS, Tween-80, and L-cysteine which are known enzyme inhibitors also strongly inhibited the pectinase from *A. fumigatus* at higher concentrations.

4. CONCLUSION

The pectinase from *Aspergillus fumigatus* exhibited some desirable properties that could be utilized in industrial processes. The optimum temperature, thermal stability, pH, substrate specificity in the presence of metal ions, and non-aqueous organic solvents make pectinase from the isolate a potential candidate for application in industrial processes. On the other hand, more researches would be needed to find out the type of pectinase from *A. fumigatus* and also establish the mechanism of action of the pectinase.

Table 5: Effect of inhibitors on *Aspergillus fumigatus* pectinase activity

Residual activity (%)	0.5 mM	1.0 mM	2.0 mM	3.0 mM	4.0 mM
Control	100.00	100.00	100.00	100.00	100.00
BME	99.59±1.23	99.56±0.98	99.58±1.21	99.54±1.05	99.17±0.48
EDTA	90.50±2.42	86.36±1.54	86.78±1.71	88.02±6.29	86.78±4.04
Triton X-100	83.47±3.26	82.75±1.45	81.36±2.14	80.17±3.91	80.58±1.98
SDS	87.19±4.32	61.57±3.71	47.52±0.33	46.69±1.53	45.87±0.75
Tween-80	96.24±2.11	69.83±3.98	39.67±0.67	38.84±0.16	38.02±0.12
L-cysteine	80.03±5.04	79.34±2.43	78.06±3.13	55.37±4.10	34.30±0.75

REFERENCES

1. Pandey A, Soccol CR, Rodriguez-Leon JA, Nigam P. Solid-State Fermentation in Biotechnology. New Delhi: Asia Tech Publishers, Inc.; 2001. p. 200-21.
2. Gupta R, Beg QK, Khan S, Chauhan B. An overview on fermentation, downstream processing and properties of microbial alkaline proteases. Appl Microbiol Biotechnol 2002;60:381-95.
3. Tochi BN, Wang Z, Xu SY, Zhang W. The influence of pectinase and pectinase/hemicellulases enzyme preparations on percentage pineapple juice recovery, particulates and sensory attributes. Pak J Nutr 2009;8:1184-9.
4. Kobayashi T, Higaki N, Yajima N, Suzumatsu A, Hagihara H, Kawai S, *et al.* Purification and properties of a galacturonic acid-releasing exopolygalacturonase from a strain of *Bacillus*. Biosci Biotechnol Biochem 2001;65:842-7.
5. Jayani RS, Saxena S, Gupta R. Microbial pectinolytic enzymes: A review. Process Biochem 2005;40:2931-44.
6. Joshi VK, Attri D. Solid state fermentation of apple pomace for the production of value added products. Nat Prod Radiance 2006;5:289-96.
7. Maria de Lourdes TM, Polizeli J, Terenzi HF. Pectinase production by *Neurospora crassa*: Purification and biochemical characterization of extracellular polygalacturonase activity. J Gen Microbiol 1991;137:1815-23.
8. Fernando TH, Jayasinghe CK, Wijesundera RL. Cell wall degrading enzyme secretion by *Colletotrichum acutatum* the causative fungus of secondary leaf fall of *Hevea brasiliensis* (rubber). Mycol Res 2001;105:195-201.
9. Yadav S, Yadav PK, Yadav D, Yadav KD. Purification and characterization of pectin lyase produced by *Aspergillus terricola* and its application in retting of natural fibers. Appl Biochem Biotechnol 2009;159:270-83.
10. Khan M, Nakkeeran E, Umesh-Kumar S. Potential application of pectinase in developing functional foods. Annu Rev Food Sci Technol 2013;4:21-34.
11. De Lorenzo G, D'Ovidio R, Cervone F. The role of polygalacturonase-inhibiting proteins (PGIPs) in defense against pathogenic fungi. Annu Rev Phytopathol 2001;39:313-35.
12. Alkorta I, Gabirsu C, Lhama MJ, Serra JL. Industrial applications of pectic enzymes: A review. Process Biochem 1998;33**:**21-8.
13. Angayarkanni J, Palaniswamy M, Murugesan S, Swaminathan K. Improvement of tea leaves fermentation with *Aspergillus* Spp. Pectinase. J Biosci Bioeng 2002;94:299-303.
14. Rasheedha AB, Kalpana MD, Gnanaprabhal GR, Pradeep BV, Palaniswamy M. Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. Indian J Sci Technol 2010;3:377-81.
15. Matti L, Jouni J, Ossi P, Ossi T, Hans ES. Industrial use of enzymes. Physiol Maint 2009;2:???.
16. Murad HA, Saleem MM. Utilization of permeate for producing exopolysaccharides from lactic acid bacteria. Mansoura Univ J Agric Sci 2001;26:2167-75.
17. Hoondal GS, Tiwari RP, Tewari R, Dahiya N, Beg QK. Microbial alkaline pectinases and their industrial applications: A review. Appl Microbiol Biotechnol 2002;59:409-18.
18. Kashyap DR, Vohra PK, Chopra S, Tewari R. Applications of pectinases in the commercial sector: A review. Bioresour Technol 2001;77:215-27.
19. Gummadi SN, Panda T. Purification and biochemical properties of microbial pectinases. A review. Process Biochem 2003;38:987-96.
20. Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. Curr Microbiol 2008;57:503-7.
21. Alexopoulos CJ, Mims CW, Blackwell M. Introductory Mycology. Vol. 4. New York: Wiley; 1996.
22. Wang G, Michailides TJ, Bostock RM. Improved detection of polygalacturonase activity due to *Mucor piriformis* with a modified dinitrosalicylic acid reagent. Phytopathology 1997;87:161-3.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
24. Silva D, Martins ES, Da Silva R, Gomes E. Pectinase production by *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural wastes and agro-industrial by-products. Braz J Microbiol 2002;33:318-24.
25. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
26. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc 1934;56:658-66.
27. O'Gorman CM, Fuller H, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 2009;457:471-4.
28. Abad A, Fernández-Molina JV, Bikandi J, Ramírez A, Margareto J, Sendino J, *et al.* What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol 2010;27:155-82.
29. Giese EC, Dekker RF, Barbosa AM. Orange bagasse as substrate for the production of pectinase and laccase by *Botryosphaeria rhodina* mamb-05 in submerged and solid state fermentation. BioResources 2008;3:335-45.
30. Ahmed SA, Mostafa FA. Utilization of orange bagasse and molokhia stalk for production of pectinase enzyme. Braz J Chem Eng 2013;30:449-56.
31. Cho SW, Lee S, Shin W. The X-ray structure of *Aspergillus aculeatus* polygalacturonase and a modeled structure of the polygalacturonase-octagalacturonate complex. J Mol Biol 2001;311:863-78.
32. Schols HA, Visser RG, Voragen AG. Pectin and Pectinase. The Netherlands: Wageningen Academic Press; 2009. p. 101-35.
33. Klug-Santner BG, Schnitzhofer W, Vrsanská M, Weber J, Agrawal PB, Nierstrasz VA, *et al.* Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. J Biotechnol 2006;121:390-401.
34. Tari C, Dogan N, Gogus N. Biochemical and thermal characterization of crude exo-polygalacturonase produced by *Aspergillus sojae*. Food Chem 2008;111**:**824-9.
35. Banu AR, Kalpana MD, Gnanaprabhal GR, Pradeep BV, Palaniswamy M. Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. Indian J Sci Technol 2010;3:377-81.
36. Hasunuma T, Fukusaki E, Kobayashi A. Methanol production is enhanced by expression of an *Aspergillus niger* pectin methylesterase in tobacco cells. J Biotechnol 2003;106:45-52.
37. Nagai M, Katsuragi T, Terashita T, Yoshikawa K, Sakai T. Purification and characterization of an endo-polygalacturonase. From *Aspergillus awamori*. Biosci Biotechnol Biochem 2000;64:1729-32.
38. Madu JO, Okonji RE, Torimir, N, Agboola FK. Purification and characterization of pectinase from *Bacillus licheniformis* obtained from a cassava waste dump. J Adv Biol 2016;8:1685-95.
39. Saad N, Briand M, Gardarin C, Briand Y, Michaud P. Production, purification and characterization of an endopolygalacturonase from *Mucor rouxii* NRRL 1894. Enzyme Microb Technol 2007;41:800-5.
40. Corredig M, Kerr W, Wicker L. Separation of thermostable pectinmethylesterase from marsh grapefruit pulp. J Agric Food Chem 2000;48:4918-23.
41. Favey S, Bourson C, Bertheau Y, Kotoujansky A, Boccara M. Purification of the acidic pectate lyase and nucleotide sequence of the corresponding gene (pelA) of *Erwinia chrysanthemi* strain 3937. J Gen Microbiol 1992;138:499-508.
42. Siddiqui MA, Pande V, Arif M. Production, purification,

and characterization of polygalacturonase from *Rhizomucor pusillus* isolated from decomposting orange peels. Enzyme Res 2012;2012:138634.
43. Phutela U, Dhuna V, Sandhu S, Chadha BS. Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposing orange peels. Braz J Microbiol 2005;36:63-9.
44. Pedrolli DB, Monteiro AC, Gomes E, Carmonal EC. Pectin and pectinases: Production, characterization and industrial application of microbial pectinolytic enzymes. Open Biotechnol J 2009;3:9-18.
45. Al-Najada AR, Al-Hindi RR, Mohamed SA. Characterization of polygalacturonases from fruit spoilage *Fusarium oxysporum* and *Aspergillus tubingensis*. Afr J Biotechnol 2012;11:8527-36.
46. Thakur BR, Singh RK, Handa AK. Chemistry and uses of pectin--a review. Crit Rev Food Sci Nutr 1997;37:47-73.
47. Hobbs HR, Thomas NR. Biocatalysis in supercritical fluids, in fluorous solvents, and under solvent-free conditions. Chem Rev 2007;107:2786.
48. Merin MG, Martin MC, Rantsior K, Cocolin L, Morata de Ambrosini VI. Characterization of pectinase activity for enology from yeasts occurring in argentine bonarda grape. Braz J Microbiol 2015;46:815-23.
49. Chen WC, Hsieh HJ, Tseng TE. Purification and characterization of a pectin lyase from *Pythium splendens* infected cucumber fruits. Bot Bull Acad Sin 1998;39:181-6.
50. Yadav S, Shastri NV. Partial purification and characterization of a pectin lyase produced by *Penicillium oxalicum* in solid-state fermentation (SSF). Indian J Biotechnol 2005;4:501-5.0

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