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Biochemical characterization of an exo-polygalacturonase of strain *Aspergillus terreus* PA3A5T and its application in fruit juice clarification

Caracterização bioquímica de exo-poligalacturonase da cepa *Aspergillus terreus* PA3A5T e sua aplicação na clarificação de sucos de frutas

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This study aimed to characterize an exo-PG from *Aspergillus terreus* PA3A5T and evaluate its application in the clarification of fruit juices. Exo-PG was obtained by submerged fermentation and it was purified by ion-exchange chromatography and gel filtration. The enzyme presented molecular mass of 95.2 kDa, with maximum activity at 70 °C in a pH of 4.0 and remaining stable in an acid pH at 60°C. $FeCl_2$, $SnCl_2$, $CoCl_2$, $ZnSO_4$, NaCl, $Al_2(SO4)_3$, $MgCl_2$, $MnCl_2$, and $CuSO_4$ ions activated the enzymatic activity, and $PbCl_2$ inhibited it completely. The value of Km was 1.83 mg mL^{-1} and Vmax was 335 U mg^{-1}. The *A. terreus* PA3A5T exo-PG proved to be very effective in the clarification process of the juices, significantly reducing viscosity and color.
Keywords: pectinase, microbial enzymes, filamentous fungi.

Este estudo teve como objetivo caracterizar um exo-PG de *Aspergillus terreus* PA3A5T e avaliar sua aplicação na clarificação de sucos de frutas. Exo-PG foi obtido por fermentação submersa e purificado por cromatografia de troca iônica e filtração em gel. A enzima apresentou massa molecular de 95,2 kDa, com atividade máxima a 70 °C em pH 4,0 e mantendo-se estável em pH ácido a 60°C. Os íons $FeCl_2$, $SnCl_2$, $CoCl_2$, $ZnSO_4$, NaCl, $Al_2(SO4)_3$, $MgCl_2$, $MnCl_2$ e $CuSO_4$ ativaram a atividade enzimática e $PbCl_2$ a inibiu completamente. O valor de Km foi de 1,83 mg mL^{-1} e Vmax foi de 335 U mg^{-1}. O *A. terreus* PA3A5T exo-PG mostrou-se muito eficaz no processo de clarificação dos sucos, reduzindo significativamente a viscosidade e a cor.
Palavras-chaves: pectinase, enzimas microbianas, fungos filamentosos.

1. INTRODUCTION

Pectin is an important structural component of plant cell walls, and pectinases are enzymes that break down pectin into simpler molecules, playing a natural role in fruit ripening. These enzymes are classified into three main categories based on their mechanism of action: protopectinases, de-esterifying (esterases), and depolymerizing (hydrolases and lyases) enzymes [1]. In the biotechnology sector, pectinases are increasingly important and are widely produced commercially, especially those of fungal origin, due to their ease of maintenance and propagation in controlled environments.

Most commercial pectinases are produced by fungi, with *Aspergillus niger* being the primary producing species [2]. Besides *A. niger*, several other fungal species are efficient in pectinase production, such as *Aspergillus awamori*, *Penicillium strictum*, *Trichoderma viride*, *Mucor piriformis*, *and Yarrowia lipolytica*. These fungi are essential in both submerged and solid-state fermentation for the production of industrially relevant products [3]. *A. terreus* is extensively studied for its effectiveness in degrading plant substances in nature and is used industrially in the production of primary metabolites: xylanase, itaconic acid, and cis-aconitic acid, and secondary metabolites: lovastatin. However, the search for an efficient pectinase from natural sources involves investigating the pectinase production capacity of *A. terreus* [4].

The applications of pectinases are continuously increasing, and the global enzyme market accounts for 25% of pectinases [5]. Pectinases have been used in various conventional industrial processes, such as textiles, processing of plant fibers, tea, coffee, juice clarification, and oil extraction [6]. Conventional juice extraction methods are energy-intensive and inefficient due to the formation of colloids caused by the high concentration of pectin. This leads to fruit juice turbidity, which also presents a market challenge. For these reasons, pectinases play a crucial role in the production and clarification of these juices, helping to prevent pectin flocculation and reduce viscosity [7].

Thus, it is crucial to highlight that, although some studies have recently documented the ability of A. terreus to secrete complex pectinolytic enzymes, this is the first study to biochemically characterize this molecule and evaluate the application of an exo-PG produced by this species in fruit juice clarification.

2. MATERIAL AND METHODS

2.1 Microorganism and cultivation conditions

The fungus *A. terreus* PA3A5T was isolated from a soil sample collected in a soybean field in the municipality of Nova Aurora, Paraná, Brazil, and is part of the fungal collection of the State University of Western Paraná. For its identification, the genomic DNA of the fungus was extracted following the methodology of White et al. (1990) [8], and the sequencing of the ITS region (internal transcribed spacer) of the ribosomal RNA was performed using the Sanger technique, conducted by Helixxa® [9, 10].

To obtain the enzymatic extract, 1 mL of the spore suspension (2.4×10^4 spores mL^{-1}) was used to inoculate Vogel medium [11], supplemented with 1% passion fruit peel as a source of carbon. The medium was then incubated at 28°C for 96 h. After the incubation period, the cultures obtained in liquid media were filtered to retain an extract free of cells and mycelium. The extract was dialyzed and used to determine the enzymatic activity and protein concentration, as well as for other experiments.

2.2 Determination of enzyme activity and protein concentration

The enzymatic activity was determined through the reaction with 3,5-dinitrosalicylic acid (DNS) described by Miller (1959) [12], using 1% citrus pectin (0.01 g mL^{-1}) in sodium acetate buffer (100 mmol L^{-1}) as a substrate. The enzymatic reaction was carried out at 70°C for 10 min. Absorbance was then measured in a spectrophotometer at 540 nm. An enzyme unit (U) was defined as the amount of enzyme needed to release one (μmol) unit of product formed under the test conditions. Protein quantification was performed according to the methodology described by Bradford (1976) [13], using bovine serum albumin (BSA) as a standard. The reaction took place at room temperature, followed by reading at 595 nm.

2.3 Purification of pectinase through chromatographic columns

The dialyzed extracellular enzyme extract was equilibrated with Tris-HCl buffer (200 mmol L^{-1}; pH 7.2) and applied to the DEAE-Sephadex anion exchange column. After washing the column with the same buffer, the proteins were eluted with a gradient of NaCl (0.05–1 mol L^{-1}). Five mL fractions were collected to determine enzyme activity and protein content. The fractions with the highest pectinolytic activity were pooled, dialyzed, lyophilized, and applied to the Sephacryl S-100 HR molecular exclusion column that was previously equilibrated with sodium acetate buffer (50 mmol L^{-1}; pH 4.0). A 1 mL fraction was collected to determine the enzymatic activity and protein content. The fractions that showed greater enzymatic activity were pooled, dialyzed, and biochemically characterized. All purification stages were carried out at 4°C.

2.4 SDS-PAGE and zymogram

The molecular weight of the purified enzyme was determined by polyacrylamide gel electrophoresis under denaturing conditions, as described by Laemmli [14], using a 6% stacking gel and 10% resolution. The molecular weight marker used in this study was PageRuler Plus Prestained from Thermo Scientific™, 10–250 kDa. After performing electrophoresis, the gel was stained with Coomassie Brilliant Blue G-250 and the molecular weight was estimated using OriginPro 9.0 software.

The enzymatic activity (zymogram) was verified under non-denaturing conditions using the methodology described by Rehm et al. (1998) [15]. After electrophoresis was performed, the gel was incubated for 30 min with 0.5% Triton X-100 at room temperature, followed by washing with distilled water and incubation for 20 min with 50 mmol L^{-1} sodium acetate buffer (pH 4.0). The gel was incubated in a 1% citrus pectin solution (0.01 g mL^{-1}) in 50 mmol L^{-1} sodium acetate buffer (pH 4.0) for 24 h, followed by staining with ruthenium red at 0.02%.

2.5 Effect of temperature and pH on enzymatic activity and stability

The optimum temperature of purified pectinase was evaluated by the dosage of reducing sugars using the Miller method [12], in temperatures that varied from 40 to 80°C. Thermal stability was determined by incubating the enzyme without substrate at temperatures of 50, 60, and 70°C for up to 120 min, followed by the dosage of the enzymatic activity.

The influence of pH on the enzymatic activity was verified through the solubilization of the reaction substrate (citrus pectin) in 0.1 mol L^{-1} citrate-phosphate buffer [16], varying the pH between 3.0 and 9.0, followed by the dosage of the enzymatic activity using the Miller method [12]. The stability at a pH was evaluated by incubating the enzyme in the absence of the substrate in the same buffer [13], varying the pH between 3.0 and 9.0, at 4°C for 24 h. After this period, the enzymatic activity was determined according to Miller [12].

2.6 Influence of metal ions on pectinase activity

The effect of metal ions on pectinase activity was evaluated by adding $Al_2(SO_4)_3$, $BaCl_2$, $CaCl_2$, $CuSO_4$, $FeCl_2$, KCl, $MgCl_2$, $MnCl_2$, NaCl, NH_4Cl, and $ZnSO_4$ to the reaction substrate (1% citrus pectin in 100 mmol L^{-1} sodium acetate buffer) at concentrations of 1 and 10 mmol L^{-1}. Ethylenediamine tetraacetic acid (EDTA) was used as a negative control for the determination of residual enzyme activity (%).

2.7 Kinetic parameters of the pectinase

The Michaelis-Menten equations were used to determine the K_m and V_{max} of the enzyme. The effect of substrate concentration on pectinase activity was evaluated using citrus pectin as substrate at various concentrations (0–2.0 mg mL^{-1}). The enzymatic assays were carried out under optimal activity conditions, and the graph was elaborated by linear regression, using OriginPro 9.0 software.

2.8 Substrate specificity

The substrate specificity analysis was conducted by incubating the enzyme with 1% (0.01 g mL^{-1}) of each substrate (citrus pectin, polygalacturonic acid, starch, Carboxymethyl cellulose (CMC), microcrystalline cellulose [Avicel®], and beechwood xylan) in sodium acetate buffer (100 mmol L^{-1}; pH 4.0) for 10 min at 70°C. Enzyme activity was determined according to Miller (1959) [12].

2.9 Analysis of the hydrolysis products

The hydrolysis products were analyzed by incubating the purified enzyme with polygalacturonic acid (0.01 g mL^{-1}) in sodium acetate buffer (100 mmol L^{-1}; pH 4.0) at 70°C for different lengths of time (10, 30, 60, and 1440 min). The reaction was stopped at 100°C for 3 min, followed by application in thin layer chromatography (TLC). The mobile phase consisted of n-butanol:ethanol:distilled water (50:25:25), and the revelation took place with 0.2% orcinol in sulfuric acid:methanol solution (1:9) at 100°C until the appearance of bands referring to hydrolysis products.

2.10 Application of pectinase in fruit juice clarification

For clarification analysis, the fruit chosen for juice extraction were the 'Bahia orange' (*Citrus sinensis*) and the 'tommy mango' (*Mangifera indica L.*). The juices were obtained by grinding the fruit pulp in a blender with the addition of water in a 4:1 ratio (fruit:water) [17]. The extracted pulp was sieved and then were added 2,5 U/mL of enzyme per mL of juice, followed by incubation for 4 h at 70°C. The experiment was carried out with 3 repetitions of treatment for each juice. As a control, the same volume of juice was incubated without the addition of enzymes. The treated juices were centrifuged at 5,000 rpm for 15 min at 4°C, and the supernatants were analyzed according to the parameters of pH, turbidity (% T660nm), and color (A420nm), using a Genesys 10S UV-Vis spectrophotometer, and compared to the control (no addition of enzyme).

3. RESULTS AND DISCUSSIONS

3.1 Purification of pectinase through chromatographic columns

At the end of the purification process, the enzyme was purified 3.3 times, with a recovery of 6% and a specific activity of 49 U mg^{-1} (Table 1). In comparison, Ázar et al. (2020) [18] obtained a purification factor of 13.3 at the end of the process, with 18.9% recovery for a pectinase from *Calonectria pteridis*; these values were higher than those found in this work.

Table 1 – Pectinase purification steps.

Purification steps	Volume (mL)	Total Protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification (fold)
Crude enzyme	60	120,0	1811	15	100	1,0
DEAE - Sephadex	20	10,2	251	25	14	1,7
Sephacryl S-100 HR	10	2,2	108	49	6	3,3

Purified pectinase was confirmed by electrophoretic homogeneity under denaturing conditions (SDS-PAGE), and it was possible to observe a single band with a relative mass of approximately 95.2 kDa (Figure 1a and 1c), which was higher than that observed by Patidar [19] for a pectinase from *A. niger*, with a relative mass of 64.5 kDa. From the zymogram (Figure 1b), the region of citrus pectin hydrolysis at the level of the enzyme indicated the pectinolytic activity of the purified enzyme.

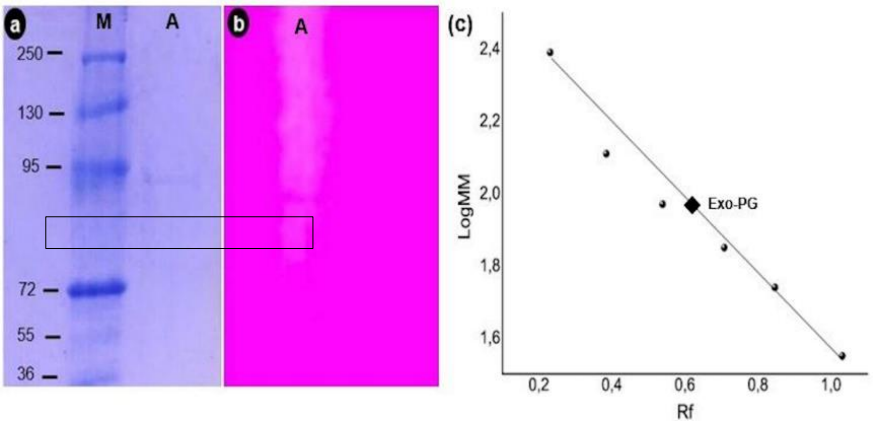

Figure 1: Electrophoresis under. (a) denaturing conditions (SDS-PAGE; (b) zymogram of the purified enzyme; (c) molecular mass of the enzyme.

3.2 Effect of temperature and pH on enzymatic activity and stability

The optimum temperature for purified pectinase was 70°C (Figure 2a), which was higher than that reported for pectinases from *Penicillium janczewskii* (45°C) [20] and *P. fellutanum* (40°C) [21]. Regarding the thermostability tests, the enzymatic activity remained above 70% in the first 30 min, being inactivated after this period. At 60°C, the enzyme maintained approximately 80% of its activity in 5 min, with a progressive reduction after this period. At 70°C, however, pectin maintained almost 100% of its activity after 5 min and approximately 60% after 30 min, being destabilized after 60 min of incubation (Figure 2b). Compared to these results, KC (2020) [22] demonstrated a reduction of approximately 50% of pectinolytic activity in 10 min of heating at 60°C; in an additional heat treatment for up to 40 min, no enzymatic activity was detected.

The thermophilic characteristic of the pectinase is advantageous, as conducting procedures at elevated temperatures with thermostable enzymes minimizes the risk of microbial contamination, which is one of the most significant benefits. Additionally, these conditions help reduce substrate viscosity, improve transfer rates, and increase solubility during reaction operations [23, 24].

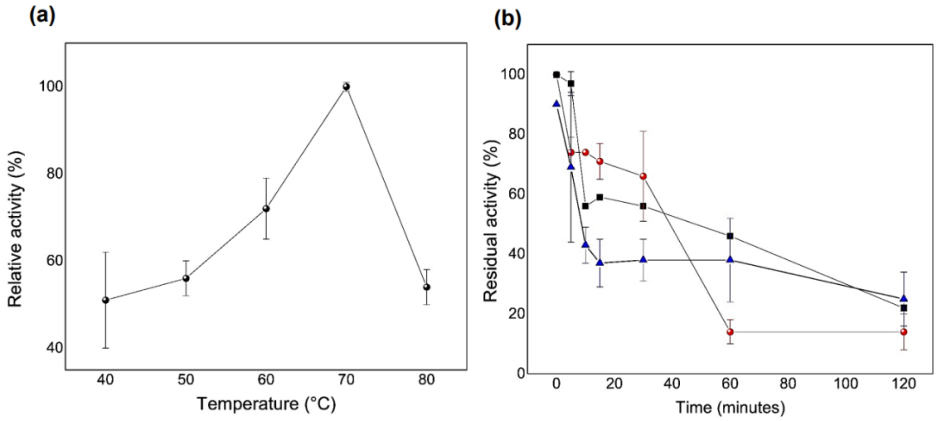

Figure 2: Effect of temperature on the activity and stability of the purified enzyme. (a) Optimum temperature; (b) Thermal stability. Symbols: (●) 50°C, (■) 60°C, (▲) 70°C.

The results obtained in the pH assays to determine enzyme activity showed that the enzyme had greater activity at a pH of 4.0 (Figure 3a). Acid pectinases were also described by Pagarra et al. (2019) [25] (pH 3.0–7.0), Shanmugavel et al. (2018) [26] (pH 4.0), and Zhong et al. (2021) [27] (pH 5.0).

Regarding the pH stability studies, pectinase was stable at a pH of 4.0 and maintained more than 80% of its activity at a pH of 5.0, and above a pH of 6.0, little activity was detected

(Figure 3b). Okonji et al. (2019) [6] obtained similar results for pectinase from *A. fumigatus*, where the enzyme remained stable at a pH of 5.0 and its activity was significantly reduced at the other tested pHs.

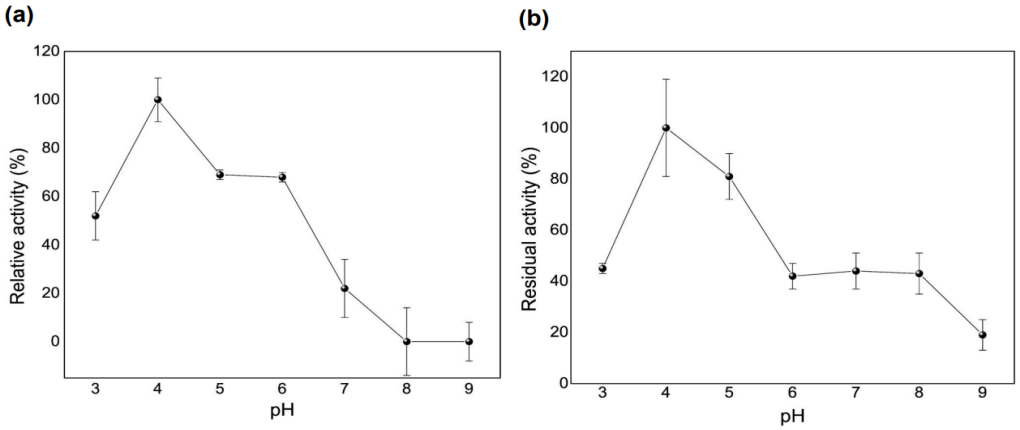

Figure 3: Effect of pH on the activity and stability of purified enzyme. (a) Optimum pH; (b) Stability at pH.

3.3 Influence of metal ions on pectinase activity

The effect of the metal ions at concentrations of 1 and 10 mmol L^{-1} on pectinase activity is represented in Table 2. The EDTA was used as a negative control for the experiment as it is a chelating agent that binds to metal ions, forming stable complexes. This removes free ions from the solution, preventing them from participating in reactions that could give false positive results in ion tests. At 1 mmol L^{-1}, the ions $FeCl_2$, $SnCl_2$, $CoCl_2$, $ZnSO_4$, NaCl, $Al_2(SO_4)_3$, $MgCl_2$, and $MnCl_2$ increased the enzymatic activity by approximately 168, 88, 85, 54, 23, 15, and 9%, respectively. At 10 mmol L^{-1}, enzymatic activation was verified in the presence of ions $FeCl_2$, $MgCl_2$, $Al_2(SO_4)_3$, and $CuSO_4$, with an increase of approximately 116, 112, 50, and 43%, respectively, and complete inactivation occurred in the presence of KCl, NH_4Cl, $CaCl_2$, $BaCl_2$, and $PbCl_2$.

Table 2: Effect of metal ions on pectinolytic activity.

Ions	Relative activity (%)*	
	1 mmol L^{-1}	10 mmol L^{-1}
EDTA	100,0 ± 0,12	100,0 ± 0,14
$FeCl_2$	267,6 ± 1,23	216,3 ± 0,17
$SnCl_2$	188,2 ± 0,78	0,00 ± 0,14
$CoCl_2$	185,3 ± 1,94	0,00 ± 0,50
$ZnSO_4$	154,5 ± 0,42	72,1 ± 0,74
NaCl	122,7 ± 0,52	ND**
$Al_2(SO_4)_3$	115,5 ± 1,11	149,6 ± 1,08
$MgCl_2$	111,7 ± 0,19	212,3 ± 0,34
$MnCl_2$	108,8 ± 1,23	0,00 ± 0,23
$CuSO_4$	98,7 ± 1,06	142,9 ± 0,39
KCl	85,2 ± 0,76	ND
NH_4Cl	84,2 ± 0,34	ND
$CaCl_2$	80,9 ± 0,44	ND
$BaCl_2$	74,1 ± 0,35	ND
$PbCl_2$	0,0 ± 0,15	ND

* Values represent the mean and standard error (±) of three independent dosages.
** ND = Not detected.

The exo-polygalacturonase from *A. terreus* PA3A5T, when tested with ions at a concentration of 10 mmol L^{-1}, exhibited greater inhibition of enzymatic activity compared to a concentration of 1 mmol L^{-1}. This occurs because higher ion concentrations generate more interactions with the enzyme's structure, causing conformational changes and loss of catalytic activity. The results with the Na^+ ion are intriguing, as the lowest tested concentration increased pectinolytic activity, while the highest concentration reduced the activity to undetectable levels. This ion probably interacts with the enzyme's active site; at low concentrations, it stabilizes the enzyme-substrate binding and enhances enzyme efficiency. However, this ion can generate a salt out effect at high concentrations, affecting the native conformation of the molecule and preventing proper substrate binding or catalysis. According to literature, monovalent cations often promote entropic balance in enzymatic reactions, resulting in more organized protein conformations that are catalytically active. However, this pattern is not common for pectinases. Several reports have indicated that monovalent metal ions could, conversely, destabilize the enzymatic activity of pectinases through selective modulation of the enzyme or enzyme-substrate complex [28].

3.4 Kinetic parameters of the pectinase

The value of Michaelis constant (K_m) for the degradation of citrus pectin by the purified enzyme was 1.83 mg mL^{-1}, which is similar to that reported for pectinase from *A. niger* (K_m = 1.6 mg mL^{-1}) [29] and *Penicillium janthinellum* (K_m = 1.74 mg mL^{-1}) [30]. However, when compared to the K_m reported for pectinases from *Aspergillus aculeatus* (K_m = 11.49 mg mL^{-1}) [31]; (K_m = 9.09 mg mL^{-1}) [32], the value found in this study was more satisfactory; the smaller the K_m, the greater the affinity of the enzyme for the substrate and the smaller the concentration of the substrate needed to reach its maximum speed for a first-order reaction.

The maximum velocity (V_{max}) found was 335 U mg^{-1}, similar to that reported for *Bacillus halodurans* (V_{max} = 351 U mg^{-1}) [33]; however, it was higher than that observed for *Achaetomium* sp. (Vmax = 98 U mg-1) [34].

3.5 Substrate specificity

The substrate specificity analysis revealed that the pectinase was capable of acting on various compounds (Table 3), with the highest catalytic activity observed on polygalacturonic acid, followed by citrus pectin, microcrystalline cellulose, and starch. This indicates its bifunctional capability to cleave different α1→4 linkages (polygalacturonic acid, pectin, and starch) and β1→4 linkages (microcrystalline cellulose).

Table 3. Substrate specificity for the pectinase purified from Aspergillus terreus PA3A5T. Greater enzymatic activities observed with polygalacturonic acid and citrus pectin.*

Substrate	Bond main	Relative activity (%)
Polygalacturonic acid	α-1,4	100 ± 0,41
Citrus pectin	α-1,4	49 ± 0,36
Microcrystalline cellulose	β-1,4	31 ± 0,61
Starch	α-1,4/ α-1,6	26 ± 0,32
Beechwood xylan	β-1,4	ND** ± 0,03
Carboxymethyl cellulose (CMC)	β-1,4	ND** ± 0,42

*Values represent the mean and standard error (±) of three independent dosages. Polygalacturonic acid was used as a control (100%) for the calculation of relative activity, as it had higher enzymatic activity.
** ND = Not detected.

Pectin is a highly complex polygalacturonic acid with various side chains, where galacturonic acid residues are predominantly linked by α1→4 bonds, similar to those found in starch, which

explains the pectinase's catalytic activity on these compounds. However, the exo-polygalacturonase from *A. terreus* PA3A5T also demonstrated the ability to cleave the β1→4 linkage found in microcrystalline cellulose, exhibiting dual catalytic action. This bifunctional characteristic has been observed in pectinases from *Hylocereus polyrhizus* [35] and *Penicillium janthinellum* [17], while that from *Bacillus subtilis* was observed only with citrus pectin [3]. The absence of activity for beechwood xylan and CMC was also reported by Cheng et al. (2016) [34] for pectinase from *Penicillium oxalicum*. The highest activity was observed with the polygalacturonic acid substrate, classifying the enzyme as a polygalacturonase.

3.6 Analysis of the hydrolysis products

The analysis of the hydrolysis products by thin layer chromatography (TLC) demonstrated that the maximum hydrolysis of polygalacturonic acid occurred after 24 h of incubation, with a single release of monogalacturonic acid as the reaction product (Figure 4).

The enzymatic behavior suggests that pectinase from *A. terreus* PA3A5T presents a mechanism of action at the terminal end of the polygalacturonic acid chain, characterizing it as an exo-polygalacturonase (EC 3.2.1.67). The same profile was observed for pectinases from *A. niger* [36] and in two polygalacturonase genes, IDSPga28-4 and IDSPga28-16, from rumen fluid cDNA [37].

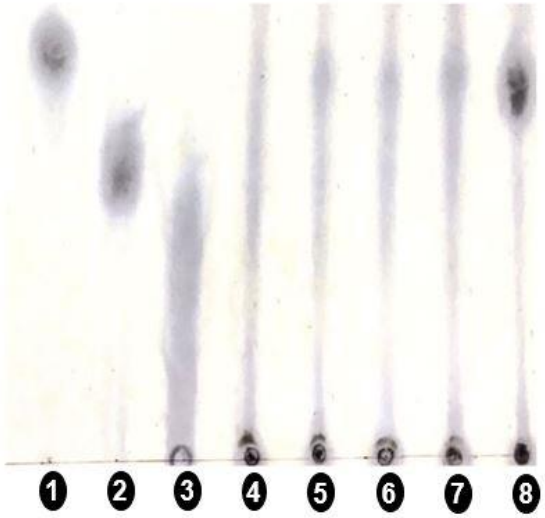

Figure 4 : Thin layer chromatography (TLC) of enzyme purified from Aspergillus terreus PA3A5T. (1) monogalacturonic acid (1 mg mL^{-1}); (2) digalacturonic acid (1 mg mL^{-1}); (3) trigalacturonic acid (1 mg mL^{-1}); (4) 1% polygalacturonic acid; (5), (6), (7), and (8) reaction incubated for 10, 30, 60 min, and 24 h, respectively.

3.7 Application of pectinase in fruit juice clarification

The effect of purified pectinase on the clarification of orange and mango juices is shown in Table 4. Clarity was expressed as transmittance (%T_{660}), and the higher the percentage, the lower the turbidity of the juice. Likewise, the greater the color reduction, the greater the percentage of absorbance (%A_{420}). Percent transmittance increased 44 and 22%, with a color reduction of 48 and 52%, for orange and mango juice, respectively. The results indicated that pectinase acted effectively in both juices, with a significant reduction in viscosity, especially for orange juice (Figure 5).

This suggests that pectinase extracted from *A. terreus* PA3A5T can be widely used in the juice clarification process due to its high hydrolysis power of the pectic substances and for its high catalytic activity in the pH range (4.0) of most fruit juices (acidic pH). The use of pectinases in

the treatment of fruit juices has been reported by many researchers in various types of fruit, such as cashew [38], pomegranate [39], papaya [40], palm [41], jamun [42], and apple [43].

Table 4: Effect of pectinase on fruit juice clarification. The data point a significant reduction in viscosity and color.*

Juice	pH initial	After treatment	$\%T_{660}$	$\%A_{420}$
Bahia orange	4,5	4,5	44	48
Tommy mango	4,4	4,4	22	52

*Data regarding control without the addition of enzymes.

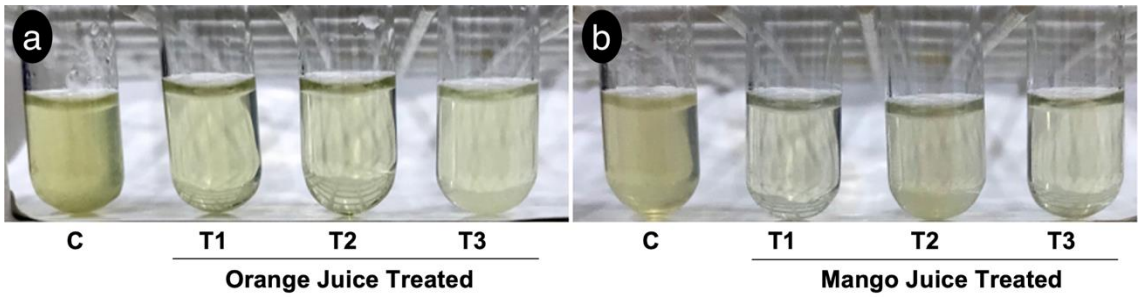

Figure 5: Clarification of (a) orange and (b) mango juice with purified exo-polygalacturonase from A. terreus PA3A5T. (C) – Control without addition of enzyme; (T1), (T2), and (T3) - Replicates of sample treatment with 2,5 U/mL of purified enzyme.

4. CONCLUSION

The exo-polygalacturonase of *A. terreus* PA3A5T can be efficiently purified by a combination of ion exchange and molecular size exclusion chromatography. The purified enzyme exhibited enhanced activity in the presence of $FeCl_2$, $SnCl_2$, $CoCl_2$, and $ZnSO_4$. Additionally, the macromolecule showed high affinity for the citrus pectin substrate, but with elevated hydrolytic activity on polygalacturonic acid, in addition to microcrystalline cellulose and starch. This hydrolytic profile indicates the enzyme's ability to cleave α-1,4, α-1,6, and β-1,4 glycosidic linkages, confirming its bifunctional nature.

Furthermore, the enzyme demonstrated thermophilic characteristics and optimal activity at pH 4.0, rendering it suitable for industrial applications in acidic environments, such as fruit juice clarification. Indeed, the enzyme effectively clarified both orange and mango juices, resulting in significant reductions in viscosity and enhancements in product color. These findings highlight the exo-polygalacturonase from *A. terreus* PA3A5T potential as a valuable tool for the food processing industry, promising improved product quality and process efficiency across diverse sectors.

5. ACKNOWLEDGMENTS

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