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Partial purification and Characterization of Exo-Polygalacturonase produced by *Aspergillusniger*strain *RA401* using Solid state Fermentation

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Abstract: In this study, an Exo-Polygalacturonase (Exo-PG) was produced from *Aspergillus niger strain RA 401* and optimized by One Variant at a Time (OVAT), high amounts of 1280 $Ug^{-1}$was achieved by optimizing the growth conditions. Homogeneity was achieved using Gel filtrationusing Sephadex G100 and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the purified resulting in a single 61.67kDa. The purified enzyme was showing maximum activity in the presence of 1% banana peel powder as a source of pectin at a temperature of 30 °C. The enzyme showed stability within the pH range of 5-7andbelow50°C. TheMichaelisconstant(K_m)andmaximumvelocity(V_{max})oftheenzymewerefound to be 0.46 $mgmL^{-1}$ and 1265µmol $(mL\ min)^{-1}$, respectively. The stability of the enzyme at a wide pH range makes it possible to have a wide range of industrial applications.

Keywords: Exo-polygalacturonase; *Aspergillusnigerstrain RA401*;purification;characterization

1. Introduction:

Pectinases are industrially important enzymes; their application were increasing day by day hence the marketing strategy requires the production of these enzymes with low costs and with optimal activities. The manufacturers will choose resources used for the production of these enzymes like carbon source and nitrogen sources with low cost and local strains with application ability. The local traits will become precious considerations for the production of such industrially important enzymes [4].

Pectinases are a group of enzymes that hybridize pectic materials, also known as pectinolytic enzymes, consist of Pectin esterase(PE), Polygalacturonase (PG),Pectin lyase(PL), and Pectate lyase (PAL)based on their mode of action. Polygalacturonases (PGs) (EC 3.2.1.67) are the hydrolytic enzymes that cleave α-1,4-glycosidic bonds that link galacturonic acid residues and are categorized into Exo-PGs (EC 3.2.1.67) acts sequentially from non-reducing end whereas, endo-PG (EC 3.2.1.15) randomly hydrolyses the α-1,4-glycosidic bonds in the polymer.[3, 17, 18, 22, 30].

Global food enzyme sales for Pectinases contribute 25%, and most of these commercial preparations are from microbes, especially from fungal sources[37, 33]. The Microbialgroups have a good capability of synthesizing pectinase enzymes and are preferred for industrial applications as they release approximately 90% of these products into the culture medium and thus can be recovered and purified[42]. Among the microbial population, production of pectinases is mostly preferred from fungi like;*Aspergillus niger*,*Penicillium*,and *Rhizopus*are generally regarded as safe strains and also yield the products into the medium which can be extracted easily from their fermentation medium and can be further purified. Techniques like Solid state Fermentation (SSF) and Submerged fermentation (SMF) can be used for the production of Polygalacturonase from fungi [16].

Pectinases are used in the food processing industry and the sales of these food enzymes contribute 25% of global sales. Most of the industrially important pectinases are produced. In literature, although several *Aspergillus* species producing pectinases for industrial applications have been reported, the election of physiologically potential strains for the production of Polygalacturonase with maximum yield remains tedious [10]. To understand an enzyme's structural and functional mechanism of action and stability,

purification and biochemical knowledge about the proteinis required. Reports show that fungal PGs.According to reports, fungal PGs are typically monomeric proteins with carbohydrate contents ranging from 5 to 85% and molecular weights between 20 and 95 kDa [18,33,16, 4].

In the current study, One Factor At a Time (OFAT) and its response for it was used to enhance the generation of an Exo-PG produced from *Aspergillus niger strain RA401* by solid-state fermentation. Following medium adjustment, Exo-PG was purified using Ammonium sulfate precipitation followed by dialysis and gel filtration. Exo- PGs physicaland chemical characteristics were described in terms of ideal pH, temperature ranges, and stability at high and low temperatures.

2. Materials and Methods:

2.1 Microorganism and Growth Conditions

Aspergillus niger strain RA401, a highly Exo-PG-producing fungus, was isolated from infected and decayed fruits and vegetables and identified at the Department of Biochemistry, Chaitanya (Deemed to be) University, Kishanpura, Hanamkonda, Telangana, India and is submitted to GenBank with Accession number MN153032. The culture was cultivated on PDA slants at 28°Cfor maintenance and sub cultured every 15 days. The culture was screened for PG production both under Submerged Fermentation Media (SMF) and solid-state fermentation (SSF) conditions based on prominent halo zone formation on the plates. Submerged fermentation media (SMF) containing $(NH_4)_2SO_4$ 0.14%, K_2HPO_4 0.6%, $KH_2PO_4$0.20%,banana peel powder as a source of pectin 1% (banana peels are dried in an oven, and powdered finely using blender), pH 5.0. The inoculum (4% v/v) was transferred to 100 mL Exo-PG production media in a 250-mL Erlenmeyer flask. Cultures were kept in an incubator shaker at 28°C and 160 rpm. The fermentations were performed under the following conditions: temperature, 28°C; aeration, 2vvm; agitation speed, 160 rpm. All experiments were performed in triplicate to ensure the trends observed were reproducible.

2.2 Production of Polygalacturonase in Submerged Fermentation(SMF)

The liquid basal medium was used along with 2% dry banana peel powder (w/w) as the sole carbon source, containing $(NH_4)_2SO_4$ 0.14%, 0.6% KH_2PO_4, and 0.01% $MgSO_4.7H_20$. At 121 °C for 15 minutes, the medium was sterilized. The inoculum (4% v/v) was transferred to 100 mLPG production media in a 250-mL Erlenmeyer flask. The flasks were carefully sealed, and they were incubated at 30°C for 5 days. Cultures were kept in an incubator shaker at 28°Cand 160 rpm. At intervals of 24, 48, 72, 96, and 120 hours, aliquots of the raw enzyme extract were taken out. The aliquots were utilized for the enzyme assay after they were filtered using Whatman No. 1 filter paper.The fermentations were performed under the following conditions: temperature, 30°C; aeration, 2vvm; agitation speed, 160 rpm. All experiments were performed in triplicate to ensure the trends observed were reproducible.

2.3 Production of Polygalacturonase Using Solid State Fermentation (SSF)

In a set of five flasks, each containing 15 g of crushed dried banana peels and 10 ml of a mineral salt solution made up of $(NH_4)_2SO_4$ 0.14%,K_2HPO_4 0.6%,KH_2PO_4 0.20%and $MgSO_4.7H_20$ 0.01%, solid-state fermentation was performed. For 40 minutes, the medium was sterilized at 121°C [24]. The test isolate was added to the flasks, which were then incubated at 28°Cfor 24, 48, 72, 96, and 120 hours. At intervals of 24, 48, 72, 96, and 120 hours,the flaks were added with 50 ml of sterile, distilled water, which was subsequently filtered. The Polygalacturonase assay was carried out using the filtrate that was collected.

2.4 Confirmation of enzyme type

The culture broth was centrifuged at 9,000 g for 15 min after 72 hours of fermentation, and the supernatant that was collected was filtered through a membrane filter (0.45 µm, Millipore). The following assays were used to identify the extracellular PG type.

2.5 Enzyme Assay

The samples' enzymatic activities were measured in units of activity per liter (U mL-1). By combining 250 L of the crude enzyme with 5.5 mL of 1% (m/v) citrus pectin in 0.05 M acetate buffer at pH 5.0 (supplemented with 1 mmol L-1 EDTA), Endo-PG activity was determined viscosimetrically. The

reaction was allowed to sit at 45 °C for 30 minutes before cooling in an ice bath. The amount of enzyme needed to reduce the initial viscosity by 50% per minute under the previously mentioned circumstances is known as a viscosimetric unit (U)[8].The 3,5-dinitro salicylic acid (DNS) assay was used to measure the release of reducing groups from polygalacturonic acid in order to assess Exo-PG activity[27, 26].
The reaction mixture, which included 0.5 mL of enzymatic extract and 0.5 mL of 0.1%polygalacturonic acid in 0.05 M acetate buffer, pH 5.0, was incubated at 45 °C for 30 min. The amount of an enzyme that releases 1 µmol of galacturonic acid per minute is considered one unit of enzymatic activity (U).

2.5 Protein determination
Using Lowry's method [23] and bovine serum albumin as a reference, the total protein content of the cell-free filtrate was determined.

2.6 Effect of Inoculum Size on Enzyme Production
By harvesting one slant in 20 ml of the sterile saline solution under aseptic circumstances, several inoculum sizes of the most powerful isolates' heavy spore suspension were made. In each fermentation flask, the inoculum sizes of 1, 2, 4,6, 8, and 10 ml were used. Polygalacturonase production was assessed for each flask at the end of the incubation period.

2.7 Effect of Carbon Sources on Enzyme Production
In order to determine the optimal carbon source for the fermentation, one percent of each of the following sugars was added to the fermentation medium: glucose, fructose, sucrose, maltose, and lactose. At the end of each fermentation procedure, enzyme activity was assessed.

2.8 Effect of Nitrogen Sources on Enzyme Production
In order to determine the optimal nitrogen source for the fermentation, nitrogen sources containing 0.14% each of NH_4Cl, $NaNO_3$, KNO_3, Peptone, Yeast Extract, and Urea were individually added to the fermentation medium. At the end of each fermentation procedure, enzyme activity was assessed.

2.9 Purification of Crude Enzyme
2.9.1 Ammonium sulfate precipitation
The cell-free filtrate (100 ml) was brought to 50% saturation by mixing with ammonium sulfate (Sigma) slowly with gentle agitation and allowed to stand for 24 h at 4°C. After the equilibration, the precipitate was removed by centrifugation (5000 rpm at 4°C for 15 min). The obtained precipitate was dissolved in 50 ml of 0.05 M sodium acetate buffer.

2.9.2Desalting by Dialysis
The precipitate was desalted using dialysis as described in [17]. Rinsing in distilled water activated the 10 cm dialysis bag. The produced precipitate was added to the bag, which had one end tied securely. To avoid any leakage, the other end of the dialysis bag was securely knotted. The dialysis bag was then suspended in a beaker containing 0.05 M sodium acetate buffer to remove ions and low molecular weight compounds that might impair the function of the enzyme. The protein fraction with Exo-PG activity was desalted overnight by dialysis at 4ºC

2.9.3 Gel filtration chromatography
Gel filtration chromatography was used to further purify the dialyzed enzyme fraction. According to Ajayi's instructions [2], a vertical glass tube chromatography column of Sephadex G-100 (Particle size, 40-120) was produced and calibrated. The Sephadex G-100 column was loaded with 30 ml of the enzyme concentration, which was then eluted with 0.05 M sodium-acetate buffer at a flow rate of 20 ml/h. Following the collection of fractions (each 5 ml), absorbance was calculated using a spectrophotometer (absorbance at 280). The polygalacturonase activity of each fraction was examined. For further analysis, the fractions with increased enzyme activity were combined.

2.9.4 Analytical electrophoresis-SDS PAGE
SDS-PAGE was used in a (10 x 8 cm) apparatus to estimate the molecular weight of the isolated enzyme.

With a 5% (w/v) polyacrylamide stacking gel and a 12% (w/v) resolving gel in Tris/glycine buffer (pH 8.3) and a molecular weight marker (14KDa-188KDa), electrophoresis was performed in a vertical slab gel equipment in a parallel lane [21].Coomassie Brilliant Blue stain was used to visualize the protein band.

2.10 Enzyme Characterization

2.10.1 Effect of pH and Stability on enzyme activity

At a constant assay temperature of 30°C, polygalacturonase activity was evaluated in relation to pH. From 4.0 to 9.0, a variety of pH values were used. Following the assay procedure reported by [27], sodium acetate (pH 4.0, 5.0), sodium citrate (pH 6.0, 7.0), and sodium phosphate were all employed as pH buffer solutions in each case (pH 8.0, 9.0).

2.10.2Effect of Temperatureand stability on enzyme activity

By incubating each reaction mixture at various temperatures (20-70°C), the ideal temperature was identified. The ratio of the maximal activity obtained at the specified temperature range to the purified polygalacturonase obtained at a specific temperature was used to express the relative activities (as percentages) [38, 11].

2.10. 3Effect of different metal ions on enzyme activity

The following ions and salts were employed to examine how different ions affect enzyme activity: EDTA, Cu^{+2}, Zn^{+2}, Mg^{+2}, Ba^{+2}, $CoCl_2$, $HgCl_2$, $Pbcl_2$, and NaCl. The reaction mixture received the specified concentrations of the ions and salts (1 mM). In 0.25 cc of 1% pectin in 0.05 M acetate buffer at pH 5.0, each metal ion salt was added. After 10 minutes of incubation at 40°C, the relative activity was tested. The relative activity of the metal ion-free control was assumed to be 100%.

2.10.4Kinetic Parameters

By monitoring the steady-state velocities of the enzyme-catalyzed reaction at various concentrations of PGA (0.5-5 g%) in 0.05M acetate buffer (pH 5.0) at 30 °C, the Km values of the isolated enzyme were determined utilizing linear regression analysis

3. Results and Discussion

3.1 Polygalacturonase Production by Aspergillus niger in SMF vs SSF

Production of polygalacturonase by *Aspergillus niger strain RA401* in submerged fermentation and solid-state utilizing banana peels as carbon source was carried and shown in Fig. 1. The results showed that 48 hours of solid-state fermentation (88.25%) and 72 hours of submerged fermentation (75.80%) had the highest levels of enzyme synthesis. The least amount of enzyme was discovered to be produced after 120 hours of fermentation in both submerged and solid-state fermentation.

3.2Confirmation of enzyme type

Exo-PG activity was shown to predominate in the *Aspergillus niger strain RA401* cell-free supernatant, with endo-PG activity being less prevalent (Fig. 2)

3.3Effect of Inoculum Size on Enzyme Production

Fig. 3 shows how the size of the inoculum affects the production of enzymes. The results showed that up to 4 ml of inoculum volume, polygalacturonase synthesis increased and then steadily dropped. The outcome demonstrates that the enzyme was produced at its maximum level when the inoculum size was 4 ml.

3.4 Effect of Carbon Source on Enzyme Production

Fig. 4 demonstrates how the carbon source affects the synthesis of polygalacturonase using banana peels as the substrate for fermentation. In the presence of fructose (1300 U/g), *Aspergillus nigerstrain RA401*produced the most polygalacturonase, followed by glucose (1000 U/g). The outcome showed that Maltose and Sucrose were the least inducers of polygalacturonase.

3.5 Effect of Nitrogen Sources on Enzyme Production

The influence of nitrogen source on the formation of polygalacturonase by *Aspergillus niger strain RA401* utilizing banana peels as substrates for fermentation is shown in Fig. 5. Our findings showed that yeast extract, among the different nitrogen sources examined, was the optimum nitrogen for the fungus to produce the enzyme, followed by KNO_3 and $NaNO_3$ in decreasing order. For NH_4Cl and Urea, polygalacturonase synthesis was low.

3.6 Purification of Polygalacturonase from *Aspergillus niger strain RA401*

Table 1 provides a summary of the purification techniques results. The outcome showed that *Aspergillus niger strain RA 401*polygalacturonase was homogeneously purified by ammonium sulfate precipitation and one-stage gel filtration on Sephadex G100. With a purification fold of 3.81, the specific activity in ammonium sulfate salting out (50% saturation) was 15.26 U/mg protein, and 31.1% recovered in precipitation proteins. Sephadex G100 gel filtration (Fig. 6) produced a yield of 12.22% and a purification fold of 4.49. The specific activity of17.96 U/mg protein and overall purity of up to 12.22-fold were achieved (Table 1).

The existence of a single protein band on SDS-polyacrylamide gel indicated the homogeneity of the purified Exo-PG and an estimate of its molar mass as a single subunit of 61.67kDa (Fig. 6).

3.6 Effect of pH on Purified Polygalacturonase Activity and Stability

Fig.7shows relative activity of Exo-PG on pH of the medium. Polygalacturonase activity and stability are affected by pH.*A. nigerstrainRA401*polygalacturonase's pH activity profile showed the greatest value at pH 5.0. The outcome showed that the activity dropped to 27% at 9.0. After 2 hours of pre-incubation, the isolated enzyme showed wide pH stability (4-7) range. Prior to pre-incubating pure enzyme for 2 hours, the initial activity was assumed to be 100%. The results showed that stability was higher at pH 5.0 (100%) and decreased as the ideal pH was exceeded. After 2 hours of storage at pH 9.0, the enzyme lost around 75% of its initial activity.

3.7 Effect of Temperature on Purified Polygalacturonase Activity and Stability

The activity and stability of polygalacturonase are temperature-dependent, its relative activity is shown in Fig. 8. Until an optimum temperature was obtained, the pure enzyme activity increased as the incubation temperature rose. Enzyme activity decreased as the temperature rose over its optimal level. At 30°C, the enzyme's activity was high. Prior to incubation at various temperatures for 1 hour, the starting activity in the thermal stability profile was assumed to be 100%. The outcome showed that after being purified, *A. niger strain RA401*polygalacturonase activity maintained a significant amount of activity for a relatively long period of time at storage temperatures (20–40°C). Between 20 and 40 degrees Celsius, the polygalacturonase maintained over 70% of its initial activity; however, after pre-incubating at 60 and 70^0C for 1 hour each, the enzyme lost its remaining activity. At 30°C (100%), the enzyme was most stable.

3.8 Effect of Metal Ions and Some Inhibitors on Purified Polygalacturonase Activity

The effect of metal ions on purified Polygalacturonase was given in Fig. 9. Data showing Mg^{+2}, Na^{+2} and, K^{+1} acts like an activator whereas Mercury, Copper, Lead showed inhibitory activity.

3.9 Kinetic Parameters

The apparent K_m value for degradation of PGA by the purified enzyme was found to be 0.46 mg mL^{-1} and the V_{max}value of 1265μmol $(mL\ min)^{-1}$ has been obtained using linear regression analysis.

4. DISCUSSION

The aim of this study was to produce and purify industrially important Polygalacturonase from fungi using a cheapersourcethatshould be easily accessible andavailable in all seasons (banana peels).Many researchers have identified this organism as a good pectin degrader. Yet there hasn't been any research comparing their Polygalacturonase producing capacities in relation to various fermentation methods. The

results of this study indicate that solid-state fermentation seems to favor the formation of polygalacturonase more than submerged fermentation with *A. nigerstrain RA401*. Enzyme production increased gradually from 24 hours of incubation to an optimum of 48 hours, after which a steady decline was noticed. When *A. nigerstrain RA401* was examined in a solid-state and submerged fermentation using banana peels as substrate, the results demonstrate a substantial increase in enzyme production during SSF (polygalacturonase). In solid-state fermentation, the optimal incubation period was found to be 48 hours, whereas, in submerged fermentation, it was found to be 72 hours. 120 hours of incubation produced the least amount of enzyme confirming the findings of [34, 36]

Polygalacturonases are hydrolytic enzymes and many microorganisms produce this enzyme among which fungi have been found to be a good producer of this enzyme. Single microorganism produces both Exo-PG and Endo-PG. Yet there hasn't been much research that confirms whether in the medium Exo-PG or Endo-PG is released. Confirmation of Enzyme type helps in the characterization of enzymesand studying the specific enzyme's physicochemical properties and to further identify steps for purification [41].

A study was done to determine the ideal inoculum size for the best enzyme production. From a 1 ml inoculum size to a 4 ml inoculum size, there was a consistent increase in enzyme synthesis, following which a reduction was seen. The ideal inoculum size for the synthesis of polygalacturonase was determined to be four milliliters (ml), which is within the range of 2 to 10 ml inoculum size previously reported for the development of bacterium pectinase from agro-industrial wastes. Ire and Vinking*et al* also reported the same for the production of Polygalacturonase from banana peels.

The optimization of carbon source on polygalacturonase production showed that glucose and fructose were better sources of carbon than sucrose, maltose, and lactose which suggests that pectinolytic microorganisms readily utilize simple sugars (glucose and fructose) rather than complex sugars. This is because the growth of pectinolytic bacteria, which in turn produces a high output of galacturonic acid from their substrates, is facilitated by simple sugars, which are an efficient carbon source. According to [34], the formation of polygalacturonase in *Geotrichumcandidum* was promoted by carbon sources other than pectin, such as galacturonic acid, fructose, and mannose. In this study optimum enzyme was produced by fructose which was also true [11].

In this study, the organisms' preference for nitrogen sources reveals that Yeast extract produced high amounts of Exo-PG, compared to KNO_3, $NaNO_3$, NH_4Cl, whereas peptone and Urea produced the least amounts. In a similar vein, [18] reported that yeast extract (YE), peptone, and ammonium chloride were shown to increase pectinase production by up to 24% when various nitrogen sources were supplied in wheat bran medium and [29] work also showedmediumenriched with yeast extract makes it the greatest inducer for Exopolygalacturonse synthesis from *Aspergillus species* as it has been noted that the inclusion of vitamins, minerals, and amino acids in yeast.

Ammonium sulfate precipitation, dialysis, and gel filtration were used to purify and concentrate the Polygalacturonase produced by *Aspergillus niger strain RA401*. Total protein and activity both decreased as a result, but specific activity increased from the crude extract to the Sephadex G100 purified fraction. The removal of impurities from the crude, which was the cause of the high total protein and enzyme activity, was thought to be the cause of this decline in protein activity. In this study, using ammonium sulfate fraction showed a yield of 31.2% and purification fold of 3.82% with a specific activity of 15.26 U/mg, and the Gel filtration fraction, polygalacturonase had an enzyme yield of 12.22% and purification fold of 4.5 with a specific activity of 17.96 U/mg. This is in line with other findings [13], which reported a yield of1.2% and purification fold with a specific activity of 15.28 U/mg with gel filtration fraction. According to [4] ammonium sulfate fractionation showed a yield of 77% and a purification fold of 7 with a specific activity of 1.4 U/mg whereas Gelfiltration fraction showed a yield of 57% with purification fold of 28 with a specific activity of 5.63 U/mg.

The purified enzyme's apparent Km value for breaking down PGA was discovered to be 0.46

mg/mL. With an endo-PG from *A. niger*, the Km value was reported to be 2.4 mg/mL[31]. Exo-PG from *A. tubingensis* with 3.2 mg/mL Km value [19]. Most of the microbial PGs' Km values fall between 0.1 and 5.0 [32, 35,42,40,25,8,].

While PG from the pathogenic fungus *Ustilagomaydis* revealed a very high Km of 57.84 [7], an exo-PG from the fungus *Penicilliumfrequentans* has a very low Km value of 0.059 [6].
This study's findings demonstrated that pH 5 was the optimal pH for polygalacturonase activity. As compared to one another, the polygalacturonase activity exhibited a substantial rise from pH 4 to pH 7 and a significant drop from pH 9 to pH 10. Several claims that polygalacturonase is an acidic pectinase were confirmed by this study. Similar findings from earlier investigations have been found [38]. Our findings are in line with the exogalacturonase from *Leucoagaricusgongylophorus* which functions best at a pH of 5.0 [1]. Exo-polygalacturonase enzyme activity showed a peak in *Monascus spp*. and *Aspergillus spp.* at pH 5.5. In the current work, the stability of the enzyme in an appropriate buffer system at 30°C was also studied. In the current investigation, the stability of the enzyme in an appropriate buffer system at 30°C was also examined. According to the findings, polygalacturonase was stable at pH values ranging from 4 to 7, but it showed an optimum at pH 5. This outcome is comparable to *Penicilliumcitrinum's* optimal pH of 5.0 and stability at 4 to 7 reported before [31].

Although there is a noticeable enzyme activity between 30°C and 50°C, the effect of temperature on enzyme activity revealed that 30°C was the ideal temperature for polygalacturonase activity. Nevertheless, polygalacturonase was more stable between 20 and 40°C, suggesting that pectinase is negatively impacted by temperature rise.

According to [14]*Aspergillus nigerNCIM 548* has a pectin lyase activity optimum temperature of 30°C. [4] noted that enzyme activity decreased at temperatures over 50°C.This study's findings demonstrated that pH 6 was the best pH for polygalacturonase activity. As compared to one another, the polygalacturonase activity exhibited a substantial rise from pH 5 to pH 6 and a significant dropfrom pH 9 to pH 10. Several claims that polygalacturonase is an acidic pectinase were confirmed by this study. Similar findings from earlier investigations have been found.

$Mg^{2+,}$ Na^{+},K^{+} Zn^{2+}, and $CoCl_2$ are preferred by polygalacturonase for metal ions, although Pb^{2+} and Cu^{2+} have an inhibitory effect on enzyme activity. This finding is similarto that of [39, 11] who found that K+, Ni^{2+}, Mn^{2+}, and Zn^{2+} from *Bacillus stearothermophilus*, *Bacillus cereus*, and *Bacillus subtilis* improved pectinase activity whereas Ni^{+} had a modest inhibitory effect on the enzyme. Similar to this, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}were seen to activate *Penicilliumitalicum* pectinase while $Cu^{2+}$and Fe^{2+}inhibited it [5].

5. CONCLUSION

By using cost-effective media made of banana peels which are considered waste, sustainability of fruit waste for the production of an acidic Exo-Polygalacturonase from *Aspergillus niger strainRA401* was generated. It was then purified using ammonium sulfate precipitation, and column chromatography i.e. Gel filtration (Sephadex G100). An Exo-PG with a molecular weight of 61.67kDa and an ideal pH and temperature of 5 and 30 °C was found. Purified PG was discovered to have a Km value of 0.46 mg/mL, respectively, while Mg+2 was found to increase the PG activity. Due to its acidic character, the potential of pure PG can be used in the clarifying of many fruit juices as the clarification of fruit juice can be attributed to the biochemical nature of the enzyme PG.

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Table. 1 Purification profile of Exo-PG produced by *A. niger strain RA401*

Purification step	Protein(mg) (A)	Enzyme activity (Units) (B)	Specific activity (U/mg) C= B/A	Fold Purification D= C/C1	% Yield E= (B/B1)*100
Crude	450	1800	4.0	-	-
Ammonium Sulphate (50%)	36.68	560	15.26	3.82	31.11
Gelfiltration (Sephadex G100)	12.25	220	17.96	7.5	10

C1: the value of C in row 1 (i.e. 4.0); B1: value of B in the row 1(i.e. 1800).

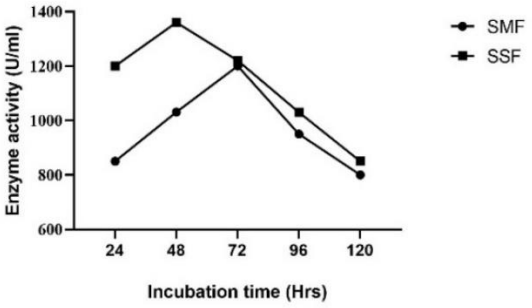

Fig. 1. Comparison of PG production by Aspergillus niger strain RA401 by SSF and SMF using banana peel powder as pectin source

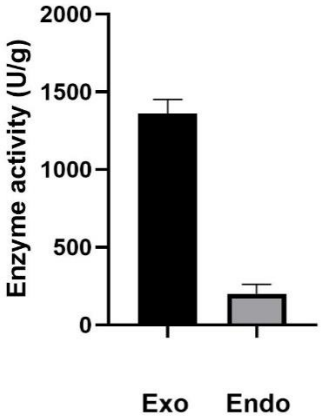

Fig. 2 Comparative enzyme activity of Exo-PG and Endo-PG

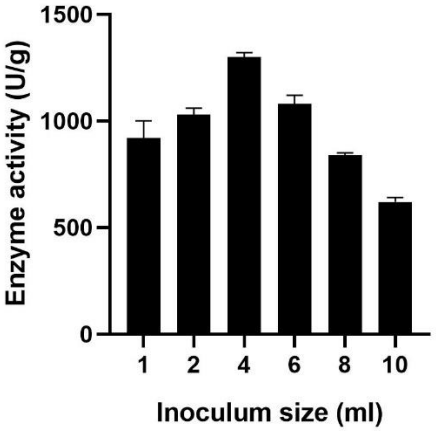

Fig. 3 Effect of inoculum size on polygalacturonase production by *Aspergillus niger strain RA401*

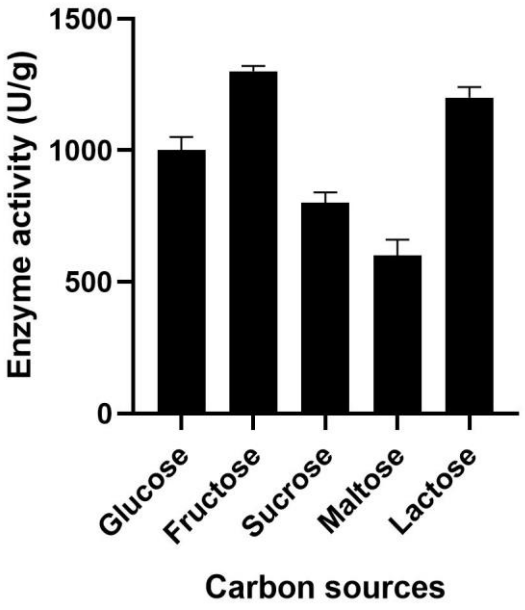

Fig. 4 Effect of carbon sources on polygalacturonase production by *Aspergillus niger strain RA401* using banana peels as substrate

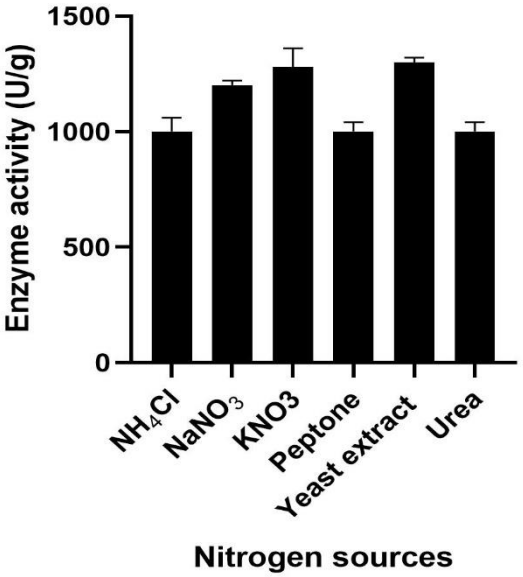

Fig. 5 Effect of Nitrogen sources on polygalacturonase production by *Aspergillus niger strain RA401* using banana peels as substrate

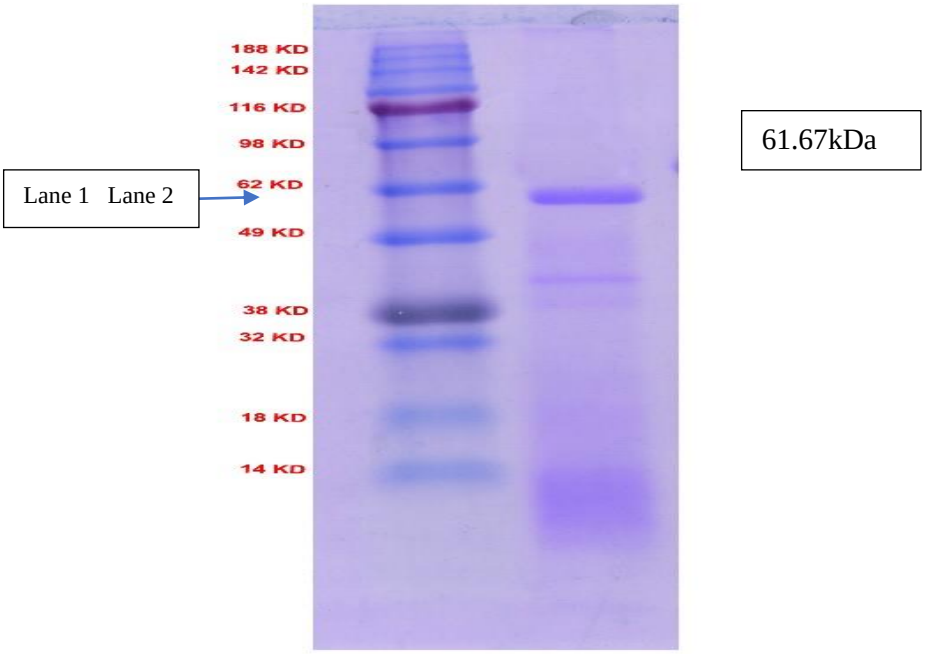

Fig. 6 SDS-PAGE: Lane 1: standard proteins
Lane 2: Purified Exo-PG

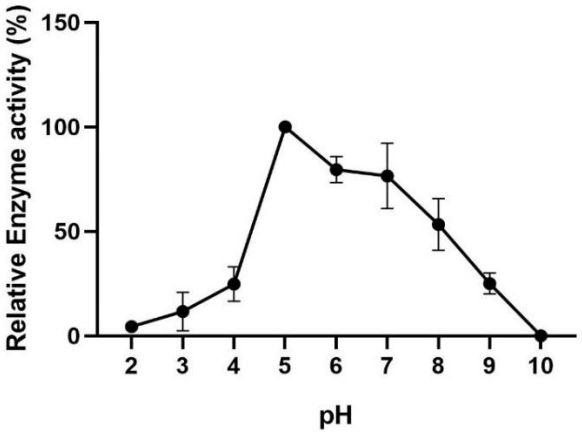

Fig. 7 Relative Exo-PG activity on pH

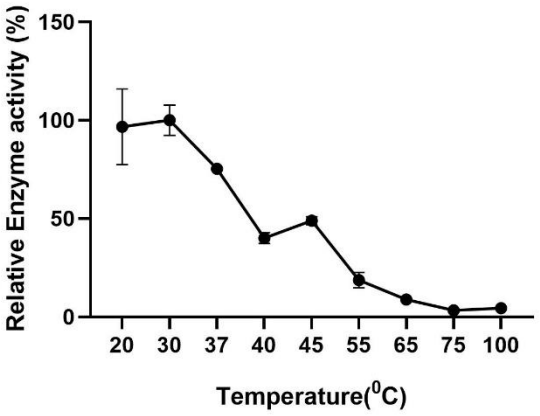

Fig. 8 Relative Exo-PG activity on temperature

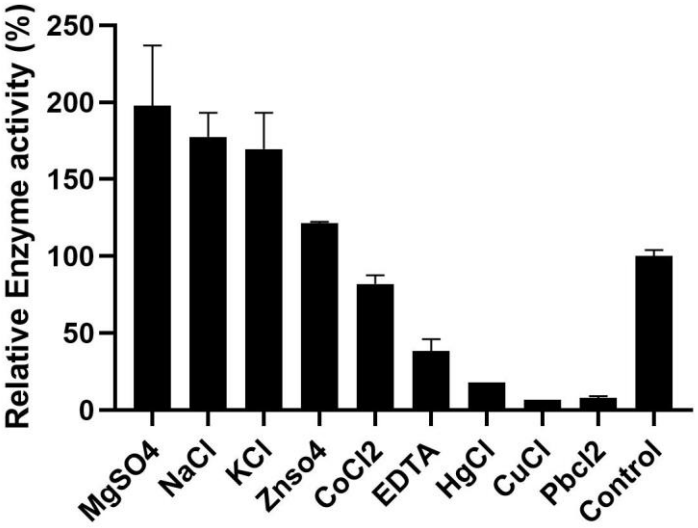

Fig. 8 Effect of metal ions on Relative Exo-PG activity

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