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Production, Purification and Comparison of Polygalacturonase from *Aspergillus flavus*, *Aspergillus niger* and *Penicillium expansum*

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ABSTRACT: The research investigated the production, purification and comparison of polygalacturonase (PG) from cassava peels degrading fungi (*Aspergillus flavus, Aspergillus niger* and *Penicillium expansum*) isolated from biodegrading cassava peels. The fungi were isolated using cassava peel agar medium and the PG was produced using the solid state fermentation process. The PG was assayed using the modified dinitrosalicyclic acid reagent method and purified by using Sephadex G-100 and Sephadex C-50. The PG were characterized by examining the effect of temperature, stability at 70 ºC, pH, substrate concentration, metal ions and EDTA. The results show that the specific activity of PG were 6.228, 6.444 and 4.569 units/mg protein for *A. flavus, A. niger* and *P. expansum* respectively while the purification fold were 75.037, 75.812 and 78.776 for the same organisms respectively. The optimum temperature and pH (45 ºC and 4.5) were the same. It was observed that the PG lost complete activity when heated at 70 ºC for 20 min. Also, the PG activity increased with increase in substrate concentration and increase in metal ions concentration (Na^+, Mg^{2+}, Ca^{2+} and K^+). However, the activity of the PG decreased with increase in heavy metal ion concentrations (Hg^{2+} and Pb^{2+}) and EDTA.

Keywords: *Aspergillus flavus,* Polygalacturonase, Cassava peel, SephadexG-100, Metal ions

Introduction

Biodegradation of agricultural waste by microorganisms produces useful end products and also reduces environmental pollution (Aransiola and Fagade, 2015). Cassava peels are agricultural waste from cassava (*Manihot esculenta* Crantz) which is usually present in large quantities in cassava processing areas where they constitute serious environmental nuisance.

Cassava peels contains pectic polymers and pectic polymers are complex polysaccharides present in the middle lamella of plant cells and are degraded by a group of enzymes known as the pectinases (Ramachandran and Kurup, 2013). Pectinases are a major means by which microorganisms invade host tissue as they attack pectin and depolymerise it by hydrolysis and trans-elimination as well as by deesterification reactions. Polygalacturonases are a group of pectinases (Ranveer, *et al*., 2005), which are hydrolytic depolymerases with endo- and exo- activities. Polygalacturonase catalyzes the hydrolytic cleavage of the polygalacturonic acid chain by introducing water across the oxygen bridge (Jayani *et al*., 2005). Endo-polygalacturonases (EC. 3.2. 1.15) attack polygalacturonic acid chains by a random hydrolysis of O-glycosyl bonds in 1,4-α-D-galactosyluronic linkages in homogalacturonans, while exo-polygalacturonases (E.C. 3.2. 1. 67) degrade polygalacturonan by hydrolysis of the glycosidic bonds from the non-reducing ends, yielding the corresponding 1,4–α–D–galacturonide and galacturonic acid (Deshmukh *et al*., 2012). Polygalacturonases are produced by various organisms such as plants (Deshmukh *et al*., 2012), bacteria (Chen *et al*.,

2014) and fungi (Cheng *et al*., 2016). Polygalacturonases are extensively used in food industries, textile processing, degumming of plant rough fibres and treatment of pectic wastewaters (Martins *et al*., 2013). Microbial polygalacturonase have been shown to play a major role in viscosity reduction and clarification of juice (Amin *et al*., 2017). Most commercial preparations of pectinases are produced from fungal sources such as strains of *Aspergillus, Pencillum* and *Erwinia* (Jayani *et al.,* 2005). Enzymes produced from the fungi, *Aspergillus, Rhizopus* and *Pencillium* are generally regarded as safe (GRAS) and they produce extracellular enzymes which can be easily recovered (Mrudula and Anitharaj, 2011). Polygalacturonase have been produced from microorganisms using solid state fermentation (SSF) or submerged fermentation (SMF), however, the solid state fermentation is more preferred because it uses various agro-industrial by products such as soy, pulps of apple, sugar beet, coffee, peels of lemon, oranges etc. (Yadav *et al*., 2014). There have been a lot of research on polygalacturonases, however, there have been limited information of polygalacturonases isolated from cassava peels hence the objective of this research is to produce, purify and compare polygalacturonase from *Aspergillus flavus, Aspergillus niger* and *Penicillium expansum* isolated from cassava peels undergoing biodegradation.

Materials and methods

Medium employed for the isolation of cassava peel degrading fungi: Cassava peel agar medium was prepared by adding 15 g of agar to 1000 ml of cassava peel extract and sterilized at 120 ºC for 15 min. Cassava peel extract was prepared by adding 50 g of ground fresh cassava peels to 1000 ml of distilled water. This was later stirred after 10 min. and filtered through cheese cloth to produce a filtrate known as the cassava peel extract.
Isolation of cassava peel degrading fungi: Sterile cassava peel agar medium (CPAM) was used for the isolation of cassava peel degrading fungi. Serial dilution of 10^{-4} - 10^{-7} of decomposing cassava peels were prepared and 1 ml. of each dilution was used to prepare CPAM plates and incubated at 28±2 ºC for 72 h. The fungal isolates from prepared plates with serial dilution of 10^{-7} of the decomposing cassava peels were subcultured into malt extract agar and yeast extract agar plates separately for the purpose of identifying the best medium for subcultivation before identification.
Characterization and identification of fungal isolates: The fungi isolates grown on malt extract agar and yeast extract agar were identified based on cultural and morphological characteristics using standard fungi identification methods outlined by Barnett and Hunter (1972), Pitt (1979) and Gilman (2001).
Cultural conditions and preparation of inoculum: Old cultures (72-96 h) were used in the preparation of the inoculum; from which a spore suspension with a spore load of appropriately 6 x 10^4 spores per ml was made. Apparently healthy freshly harvested cassava tubers were washed and peeled. The peels were further washed with tap water. Cores of cassava peels of 3mm thickness were made with a cork borer to expose more surface area for fungal attack. The cassava peel discs were surface sterilized in 3% w/v sodium hypochlorite solution for 1hr. The cassava peels discs were rinsed with six changes of sterile distilled water to remove residual effect of the sodium hypochlorite. Twenty grams of cassava peel discs were weighed out and transferred into 250 ml Erlenmeyer flasks containing 10 ml of sterile distilled water for solid state fermentation. Each flask was inoculated with 1ml of the spore suspension and incubated at room temperature for 8 days. The experimental set ups were examined daily for fungal growth and degradation of the cassava peel discs.
Polygalacturonase extraction: Daily analysis for the detection of enzyme activity commenced as soon as fungal growth was noticed (48 h). Three flasks, each of *Aspergillus niger, Aspergillus flavus* and *Pencillium expansum* were analyzed daily. The cassava peel discs in each flask were chilled for 20min and homogenized with cooled liquid extract solution (1:1 w/v). The extract solution consists 0.5 M NaCl containing 5 mM of NaN_3 to prevent microbial contamination. The homogenate from each flask was clarified by passing it through glass fibre filter (Whatman G f/A). Each filtrate was analyzed for polygalacturonase. The pH and protein content of the filtrate (crude enzyme) were determined. After the peak of enzyme production was reached, some daily analyses were additionally carried out to confirm the established peak.
Enzyme assay for polygalacturonase: Polygalacturonase enzyme activity was determined by measuring the amount of reducing sugar released in the reaction mixture. The reaction mixture was made up of 1ml of 0.4 % w/v pectin (Sigma) in 0.1M citrate phosphate buffer pH 5.0 and 0.5ml of the crude enzyme solution. This was incubated at 37 ºC for 3 h. The reducing sugars released into the reaction mixture were determined by the modified dinitrosalicylic acid reagent method (Miller, 1959). One unit of polygalacturonase activity is the amount of enzyme in 1ml of the reaction mixture that liberated reducing sugar equivalent to 1mg galacturonic acid per minute under the specified conditions of the reaction.

Partial purification of polygalacturonase

Ammonium sulphate precipitation: The crude enzyme was precipitated with 90 % ammonium sulphate solution at 4ºC for 24 h. The resulting precipitate was collected by centrifugation at 20,000 x g for 30 min. The precipitate was dissolved in 10ml of citrate phosphate buffer (pH 6.0) and dialyzed against two changes of the same buffer for 24 h. The protein content of the enzyme was determined by the method of Lowry *et al* (1951).

Further purification using SephadexG-100 and SephadexC-50: 10 ml of the dialyzed enzyme concentrate was applied to the SephadexG-100 column and eluted with 0.05 M citrate phosphate buffer containing 5 mM of NaN_3 as described by Olutiola and Ayres (1973). Three fractions (5 ml per tube) were collected and the protein content in each was determined by the method of Lowry *et al* (1951). The fractions were further analyzed for enzyme activities. The fraction of the enzyme which showed highest enzyme activity after gel filtration was concentrated in a rotary evaporator (BuchiRotavapor-R) at 28 $\pm$ 2 ºC. The enzyme concentration was made up to 10ml by adding 0.2M citrate phosphate buffer (pH.6.0) and applied to the column of SephadexC-50. This was then eluted with the same buffer containing 0.2MKCl. Fractions of 5ml per tube were collected and their protein content measured. The enzyme activity was also determined.

Enzyme characterization: The effect of some physicochemical parameters on the activities of the purified (SP C-50 fraction) enzyme was examined.

Effect of temperature: The effect of temperature on the activity of polygalacturonase was investigated by incubating the reaction mixture at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ºC for 3 h. Enzyme activity was determined after incubation for each of the above temperature.

Effect of heating at 70 ºC: The effect of heat on the stability of the enzyme was examined. Samples of the purified enzymes were heated at 70 ºC for different period of time (0, 5, 10, 15, 20, 25, 30 and 35 min respectively) the activity of the heated enzyme was measured by incubating the enzyme substrate mixture at 35 ºC for 3 h.

Effect of pH: The effect of pH on the activity of the polygalacturonase enzyme was examined.Substrate (0.4% pectin) with pH ranging from 3.0 to 9.0 was prepared. Citrate phosphate buffer (0.02M) was used to prepare substrate of pH 3.0, 3.5, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 whereas 0.2 M Tris-HCl buffers were used to prepare substrates of pH 7.5, 8.0, 8.5 and 9.0. The pH of the substrate was adjusted where necessary with 0.01M HCl and 0.01M NaOH.

Effect of substrate concentration: The reaction speed V_{max} and K_m were determined for the PG enzyme by varying the substrate concentration from 2-18mg/ml and plotting substrate/velocity as function of substrate concentration (Lineweaver and Burk, 1934).

Effect of metal ions: The effect of some metal ions at various concentrations on the activity of polygalacturonase was investigated. The substrate was incubated with each test metal ion at 4 ºC for 3hrs before being employed in enzyme assays. Different concentrations (5 - 40 mg/ml) of Na+, K+, Ca^{2+} and Mg^{2+} (sodium chloride, potassium chloride, calcium chloride and magnesium chloride) were employed for the investigation. Concentrations of 1-10 mg/ml were employed for mercury II chloride and lead II chloride (Hg^{2+} and Pb^{2+}) ions.

Effect of Ethylene diaminetetraacetic acid: The effect of ethylene diaminetetraacetic acid at a concentration of 1-10 mg/ml on the activity of the polygalacturonase was determined. The substrate was incubated initially with ethylene diamine tetra-acetic acid at 4 ºC for 3 h before they were employed in enzyme assay. All the analyses were performed in triplicate. The mean value was taken as the result.

Results and discussion

The cassava peels were prepared and fungi were isolated. The identified fungi isolates in the cassava peels were identified to be *Aspergillus flavus, Aspergillus niger and Penicillium expansum*. These were established based on their cultural and morphological characteristics. These results are similar to that reported by Deshmukh *et al.,* 2012.

The peck of polygalacturonase activity at day 5 with the associated total protein and pH is presented in Table 1. Polygalacturonase activities was at its peck on the fifth day of incubation. This result was similar to that reported by Padma *et al.* (2012), where polygalacturonase production by *Aspergillus awamori* reached its optimal activity on the fourth day.

The purification profiles used in the production of purified polygalacturonase from *Aspergillus flavus, Aspergillus niger* and *Penicillium expansum* are presented in Tables 2, 3 and 4. The polygalacturonase activity in the crude enzymes from cassava peels degraded by *A. flavus, A. niger* and *P. expansum* were 0.718, 0.617 and 0.406 units

respectively (Tables 2, 3, 4). This indicates that the polygalacturonase activity increased in the order *A. flavus>A. niger>P. expansum*. This order was also maintained after the purification processes.

Table 1: Polygalacturonase activity, total protein and pH at the peck of enzyme production

Isolated fungus	Total polygalacturonase activity (Unit)	Total protein (mg)	pH
Aspergillus flavus	0.718	8.68	4.5
Aspergillus niger	0.617	7.280	4.5
Penicillium expansum	0.406	6.990	4.5

Table 2: Purification profile of polygalacturonase obtained from cassava peel degraded by *Aspergillus flavus*

Fraction	Total polygalacturonase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield %	Purification fold
Crude	0.718	8.68	0.083	100.000	1.000
$(NH_4)_2SO_4$	0.632	1.206	0.524	88.220	6.314
G – 100	0.559	0.214	2.612	77.855	31.471
SP C – 50	0.355	0.057	6.228	49.443	75.037

Table 3: Purification profile of polygalacturonase obtained from cassava peel degraded by *Aspergillus niger*

Fraction	Total polygalacturonase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield (%)	Purification fold
Crude	0.617	7.280	0.085	100.00	1.000
$(NH_4)_2SO_4$	0.560	1.103	0.508	90.760	5.976
G – 100	0.472	0.190	2.484	76.499	29.224
SP C – 50	0.348	0.054	6.444	56.402	75.812

Table 4: Purification profile of polygalacturonase obtained from cassava peels degraded by *Penicillium expansum*

Fraction	Total polygalacturonase activity (units)	Total protein (mg)	Specific activity (unit/mg protein)	Yield (%)	Purification fold
Crude	0.406	6.990	0.058	100.000	1.000
$(NH_4)_2SO_4$	0.369	0.975	0.379	90.886	6.535
G – 100	0.295	0.165	1.788	72.660	30.828
SP C – 50	0.233	0.051	4.569	57.389	78.776

However, the polygalacturonase activity after purification was a function of the purification process. The polygalacturonase activity was higher in ammonium sulphate purification process with the specific activity, percentage yield and purification fold varying from 0.524 - 0.379 unit/mg protein, 88.220 - 90.88 % and 6.314 - 6.535 respectively.

Similarly, the lowest polygalacturonase activity was observed in Sephadex C-50 purification process. The specific activity, percentage yield and purification fold varied from 6.228 - 4.569 unit/mg protein, 49.443-57 - 389 % and 75.037 - 78.776 respectively. However, the polygalacturonase activities from Sephadex G-100 purification process gave intermediate values but the pattern was the same with those of ammonium sulphate and Sephadex C-50. Martins et al. (2013) reported a higher specific activity of 60.0 unit/mg protein for the polygalacturonase produced by a thermophilic fungus *Thermoascuis aurantiacus*

The properties of the polygalacturonase from *Aspergillus flavus, Aspergillus niger and Penicillium expansum* were studied by observing the effects of varying temperature, heating period, pH, substrate concentration, metal ions concentration, heavy metal ions concentration and EDTA concentration. The results are presented in Figures 1 to 9.

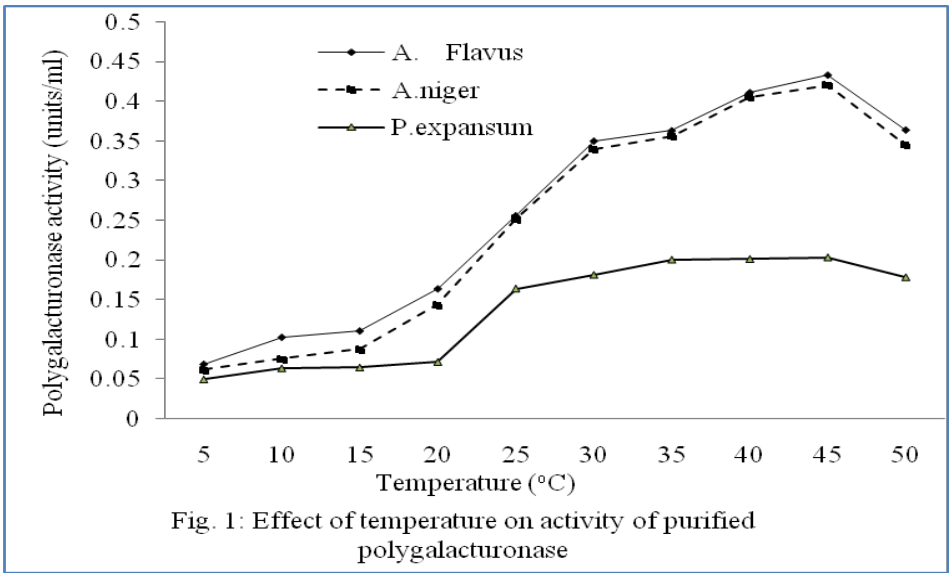

Fig. 1: Effect of temperature on activity of purified polygalacturonase

The effects of temperature on the activity of polygalacturonase are presented in Figure 1. The activity of polygalacturonase from *A. flavus* increased from 0.069 - 0.433 unit/ml as the temperature increased from 5 - 45 ºC. However, the polygalacturonase activity decreased drastically after 45 ºC, an indication that the optimum temperature for polygalacturonase from *A. flavus* is 45 ºC. Similar trends were observed for polygalacturonase activities from *A. niger* and *P. expansum*. The order of polygalacturonase activity with varied temperature was *A. flavus>A. niger>P. expansum*. Similarly, Deshmukh*et al*. (2012) reported a temperature optimum of 46 ºC for *Aspergillus niger* and *Aspergillus flavus.* Anand *et al.* (2017) reported a higher temperature optimum of 50 ºC for polygalacturonase obtained from *Aspergillus niger*. However, several fungal polygalacturonases are known to exhibit temperature optima between 40 - 60 ºC (Thakur *et al.*, 2010).

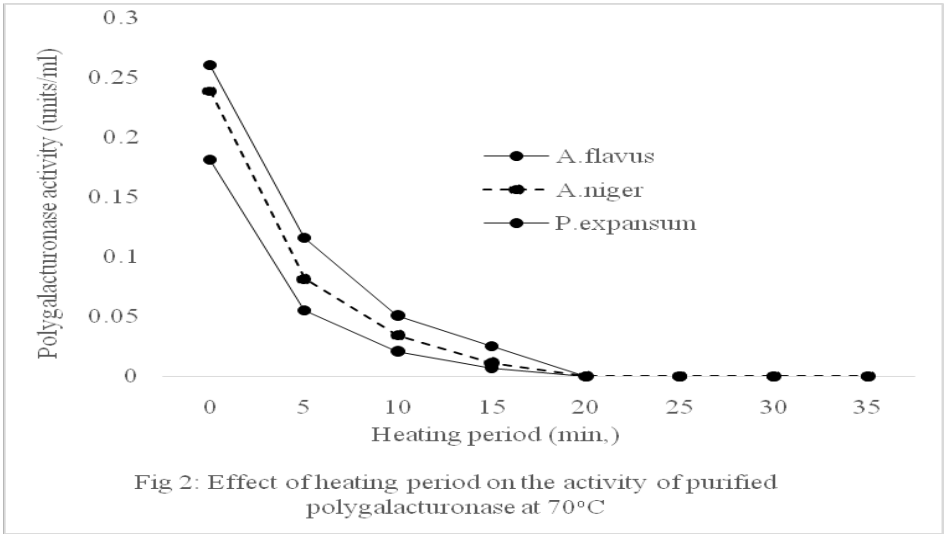

Fig 2: Effect of heating period on the activity of purified polygalacturonase at 70°C

Figure 2 shows the effect of heating period on the activity of polygalacturonase at 70 ºC. The polygalacturonase activity for *A. flavus* drastically decreased from 0.260 to 0.000 as the heating period increased from 0 to 15 min. Similar trends were also observed for *A. niger* and *P. expansum*. Beyond 15 min, the polygalacturonase activity from *A. flavus, A. niger* and *P. expansum* were zero, that is, no activity was detected for polygalacturonase. This

shows that high temperature not only decreased polygalacturonase activity but deactivated it completely after 15 minutes. The order for decrease in polygalacturonase activity when heated for 15 minutes was *A. flavus>A. niger* and *P. expansum.* Kusuma and Reddy (2014) reported that heating pectinases at temperatures above 70 ºC resulted in decrease in its activity due to partial denaturation. Also, Roosdiana *et al.* (2013) stated that high temperatures produce conformational changes in the enzymes and as a result it becomes difficult for the substrate to enter the active site and enzymatic reaction will not occur easily. Besides, Daniel *et al*. (1996) reported that heating enzymes at high temperatures often resulted in their inactivation.

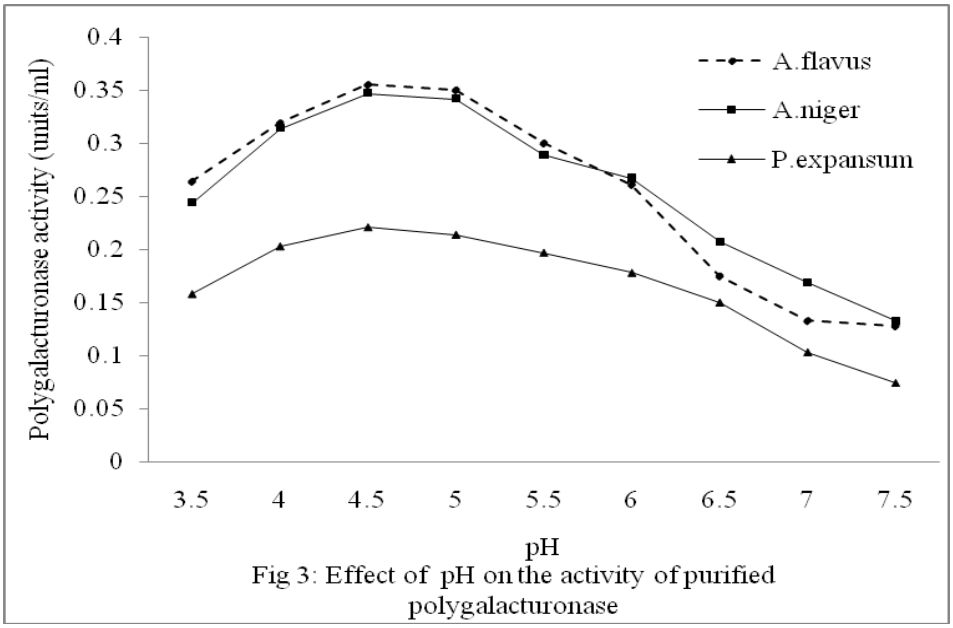

Fig 3: Effect of pH on the activity of purified polygalacturonase

The effect of pH on the activity of polygalacturonase is shown in Figure 3. The polygalacturonase from *A. flavus* increased from 0.264 to 0.355 unit/ml as pH increased from 3.5 to 4.5. Further increase in pH from 4.5 to 7.5 resulted in gradual decrease of polygalacturonase activity from 0.355 to 0.128 unit/ml. The patterns for *A. niger* and *P. expansum* were similar to that of *A. flavus*. However, the order was *A. flavus>A. niger* and *P. expansum*. The results indicate that the optimum pH for polygalacturonase activity was 4.5. Anand *et al*., (2017) reported an optimum pH of 4.0 for the polygalacturonase obtained from *Aspergillus niger*. Similar pH optimum of 4.0 was reported for exo-polygalacturonase purified from *Aspergillus sojae* and *Paecilemyces variotii* (de Lima Damasio *et al.,* 2010). However, Deshmukh *et al*., (2012) reported an optimum pH of 5.5 for *A. niger* and *A. orgzae* and Pasha *et al*., (2013) reported an optimum pH of 5.5 for the polygalacturonase from *Aspergillus foetidus*. Yadev *et al*., (2012) reported that majority of fungal polygalacturonases have their pH optimal in an acidic range.

Figure 4 indicates effect of substrate concentration on the activity of polygalacturonase. The polygalacturonase from *A. flavus* increased from 0.272 to 0.658 unit/ml as the substrate concentration increased from 2 to 12mg/ml. Further increase in substrate concentration from 12 to 18mg/ml resulted in no increase in polygalacturonase activity. Similar trends were observed for *A. niger* and *P. expansum*. The order was also similar to that of temperature and pH earlier reported. Ramachandran and Kurup (2013) reported a high polygalacturonase activity of 6.060 unit/g of dry weight of substance when wheat bran and pectin were used as substrate for *Penicillium citrimum* activity and a higher value of 14.511 unit/g of dry weight of substance when wheat brain and glucose were used as substrates.

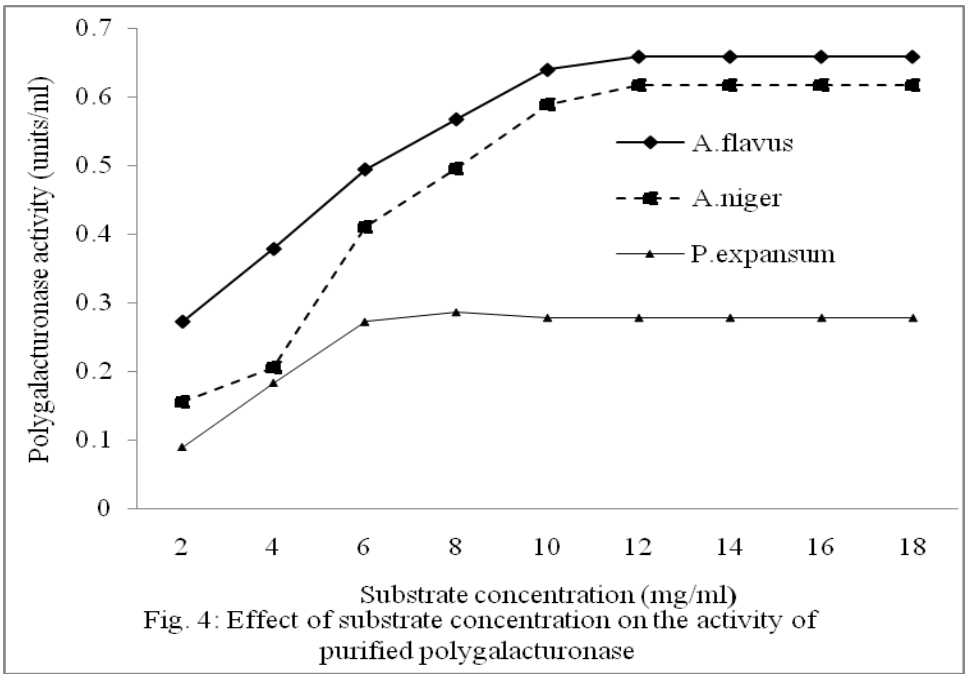

Fig. 4: Effect of substrate concentration on the activity of purified polygalacturonase

The V_{max} and K_m for polygalacturonase activity from *A. flavus, A. niger and P. expansum* are presented in Figs. 5a, 5b and 5c respectively. The figures show the Lineweaver Burk plots for the hydrolysis of pectin by the purified polygalacturonase. The apparent K_m for the hydrolysis of pectin was in the order of *A. flavus* (4.8) <*P. expansum* (7.69) <*A. niger* (13.33) mmol/ml. The V_{max} values for *A. flavus, A. niger* and *P. expansum* are 84.43, 55.56 and 131.58 unit/mg respectively. *A flavus* had the lowest K_m value, this shows that *A. flavus* had the highest affinity for the pectin when compared to *P. expansum* and *A. niger* with higher K_m. Anand *et al*., (2017) reported a lower K_m value of 2.3mg/ml for the polygalacturonase of *Aspergillus niger* while Castruita-Dominguez *et al*., (2014) reported a very high K_m value of 57.844 mg/ml for the pathogenic fungus *Ustilago maydis*. However, Chen *et al*., (2014) reported that the K_m values of most of the microbial polygalacturonases are in the range of 0.1 – 5.0 mmol/ml.

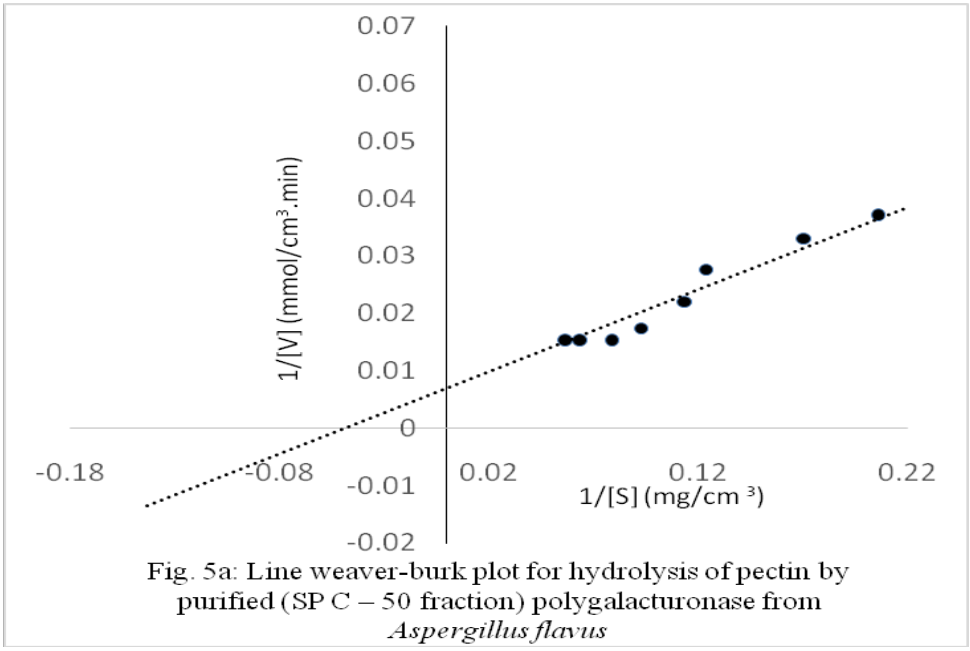

Fig. 5a: Line weaver-burk plot for hydrolysis of pectin by purified (SP C – 50 fraction) polygalacturonase from *Aspergillus flavus*

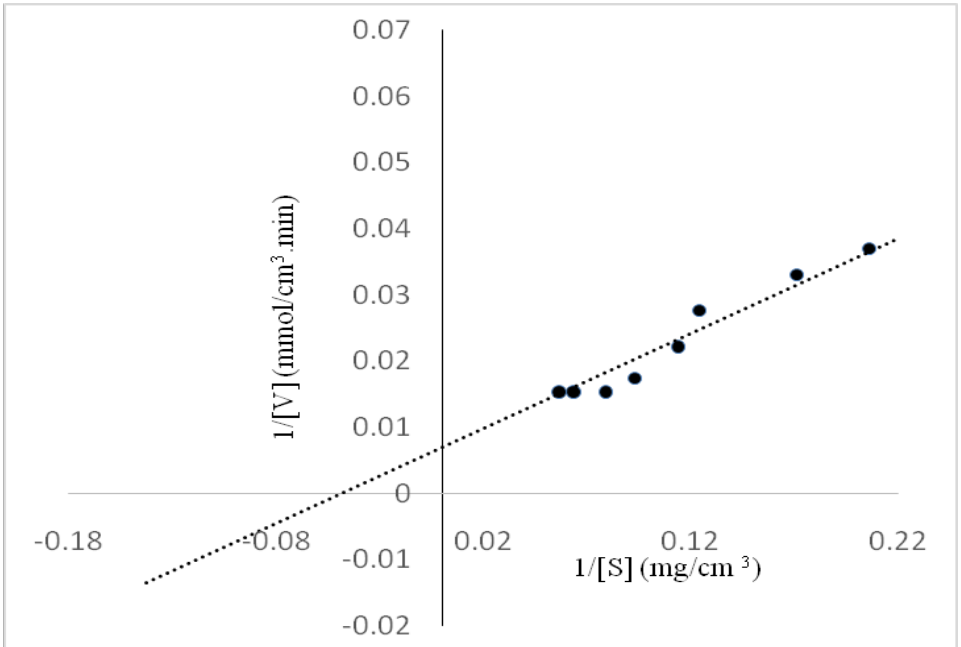

Fig. 5b: Line weaver-burk plot for hydrolysis of pectin by purified (SP C – 50 fraction) polygalacturonase from *Aspergillus niger*

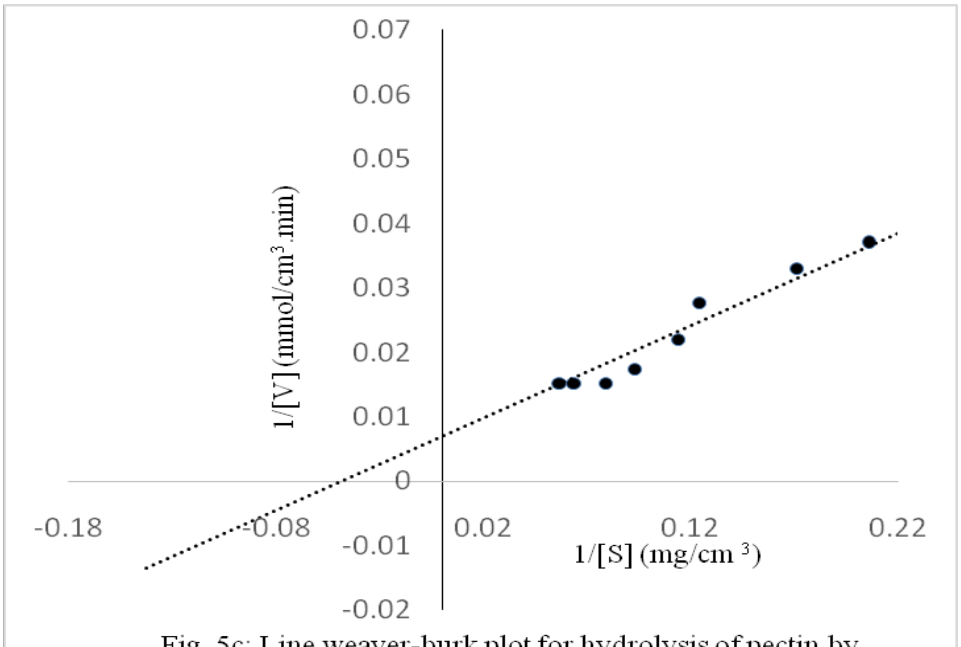

Fig. 5c: Line weaver-burk plot for hydrolysis of pectin by purified (SP C – 50 fraction) polygalacturonase from *Penicillium expansum*

The effect of metals ions (K^+, Mg^{2+}, Na^+ and Ca^{2+}) concentration on the activity of polygalacturonase are shown in Figs. 6 and 7. The results show that as the K^+ concentration increased from 0 to 30 mg/ml, the polygalacturonase activity from *A. flavus* increased from 0.364 to 0.489 unit/ml. There were no further increase in polygalacturonase activity as the K^+ concentration increased to 40 mg/ml. Similar trend was observed for Mg^{2+} and in both ions, it was *A. flavus* > *A. niger* > *P. expansum*. However, there was a slight variation in pattern for Na^+ and Ca^{2+}. Generally, the metal ions enhanced the activities of polygalacturonase irrespective of the source. The order was $Na^+ < Ca^{2+} < K^+ < Mg^{2+}$. Anand *et al*., (2017) observed a slight activation in the activity of polygalacturonase when Mg^{2+} and Ca^{2+} ions were introduced. Similarly, Banu, *et al*., (2010) reported the enhancement of pectinase activity produced by *P. chrysegenum* when Ca^{2+} was employed. Also, Yoon *et al*., (1994) reported the enhancement of polygalacturonase activity in the presence of Na^+ and Mg^{2+}. Beg and Gupta (2003) explained that the discrepancy in the divalent metal ions (Ca^{2+} and Mg^{2+}) preference suggests that the enzyme might heave differential flexibility in the active site. Ca^{2+} and Mg^{2+} have also been suspected to play a vital role in maintaining the active confirmation of alkaline endopolygalacturonases to stimulate their activity (Li, *et al*., 2008).

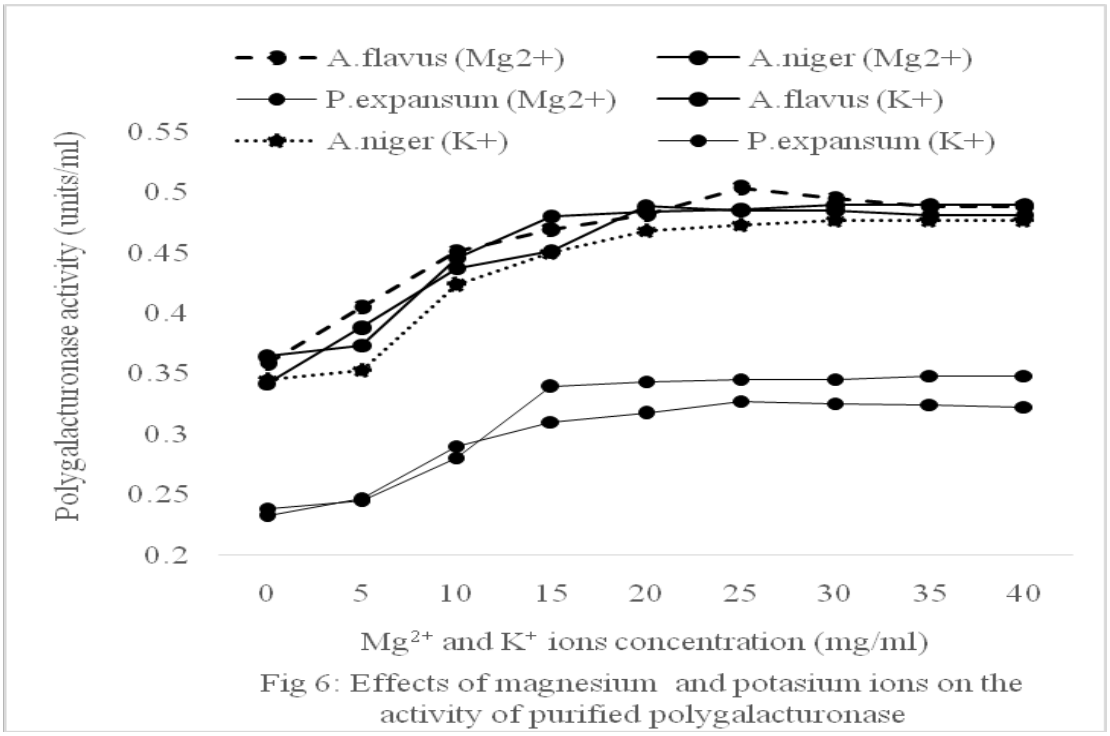

Fig 6: Effects of magnesium and potasium ions on the activity of purified polygalacturonase

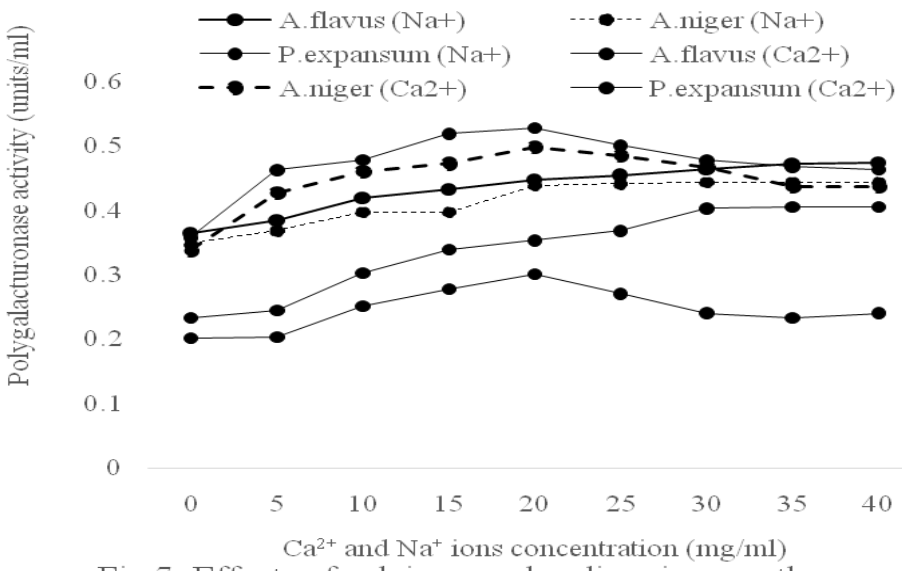

Fig 7: Effects of calcium and sodium ions on the activity of purified polygalacturonase

Figure 8 shows the effect of heavy metal ions (Hg^{2+} and Pb^{2+}) concentration on the activity of polygalacturonase. The polygalacturonase activity from *A. flavus* drastically decreased from 0.371 to 0.030 unit/ml as the Hg^{2+} concentration increased from 0 to 7mg/ml. Further increase in Hg^{2+} concentration from 7 to 10 mg/ml resulted in deactivation of the enzyme (i.e. loss of activity). The effect of Pb^{2+} on polygalacturonase activity was similar to that of Hg^{2+}. However, the decrease was gradual and beyond 7 mg/ml Pb^{2+}concentration, it was observed that for Pb^{2+} the polygalacturonase was not deactivated despite its low activity. Also, the order of activity was *P. expansum*>*A. niger* >*A. flavus*. The effect of heavy metals on the activity of polygalacturonase was retarding and the polygalacturonase from *P. expansum* was least affected at low heavy metal concentration. The heavy metal ions (Hg^{2+} and Pb^{2+}) used in this investigation were inhibitory at all concentrations to the polygalacturonase.

Banu *et al*. (2010) reported the inhibition of the pectinase activity of *P. chrysogeum* by $HgCl_2$. Similarly, Martins *et al*. (2013) reported the inhibition of polygalacturonase activity from *Thermoascuis aurantiacus* by mercury ion.

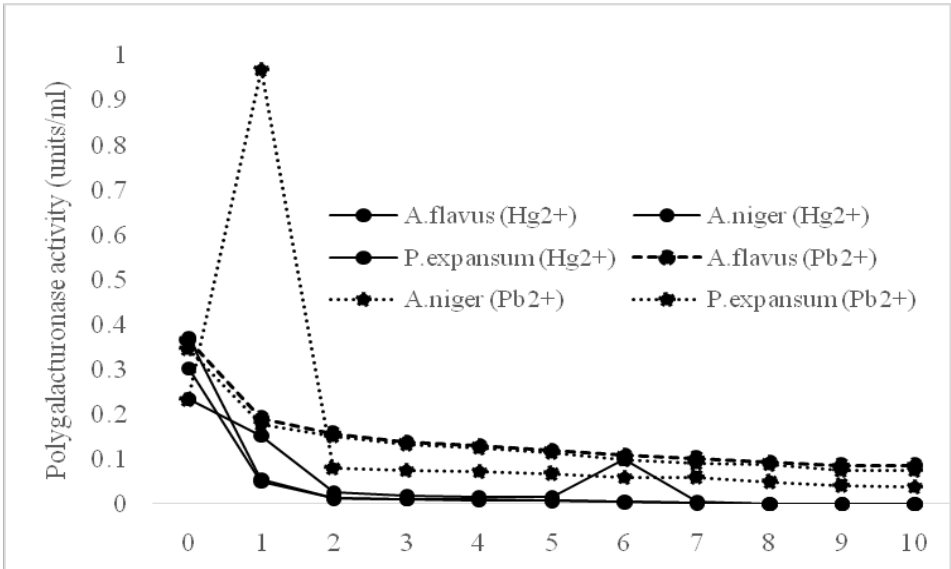

Fig 8: Effects of heavy matals ions on the activity of purified polygalacturonase

Figure 9 shows the effect of EDTA on the activity of polygalacturonase. It was observed that all concentrations of EDTA used inhibited the activities of polygalacturonase produced by *A. flavus, A. niger* and *P. expansum*. Banu *et al.* (2010) also reported the inhibition of *P. chrysogenum* polygalacturonase by EDTA.

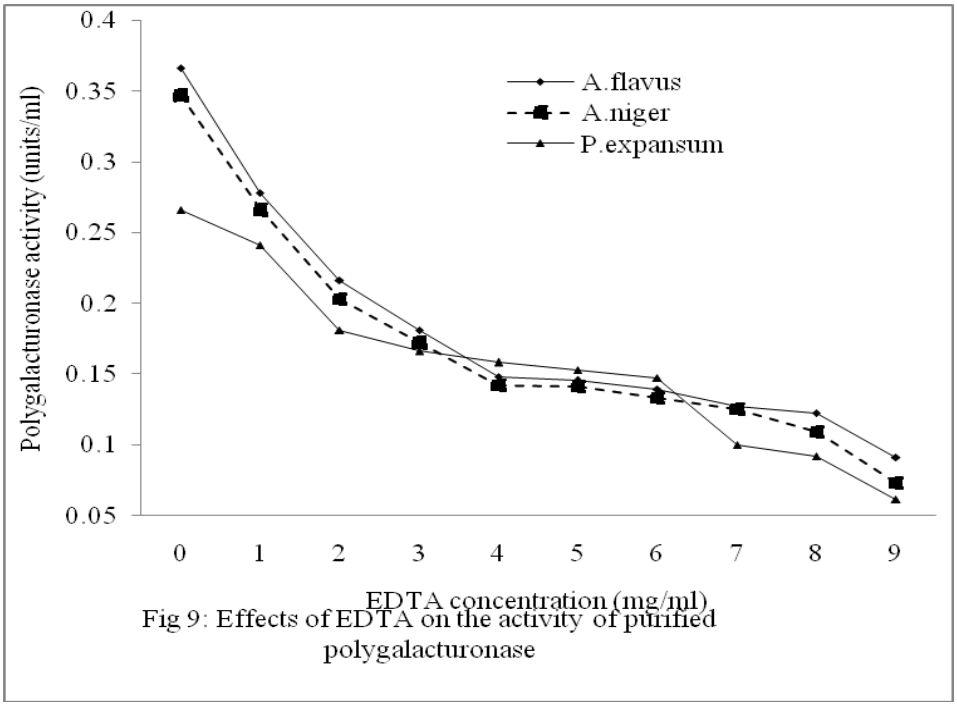

Fig 9: Effects of EDTA on the activity of purified polygalacturonase

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