International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Production of pectinase from strains of *Aspergillus niger* using corn pomace by solid state fermentation (SSF)

[1]Famotemi, A. C*; [1]Lawal, A. K. , [1]Dike, E.N., [1]Olatope, S. O.A., [1]Shittu K.A., [1]Itoandon, E.E., [1]Kehinde, M. O., [1]Orji, F. A. and [2]Elemo, G. N.

[1]Enzyme Technology Division, Department of Biotechnology,
[2]Food Technology, Federal Institute of Industrial Research, Oshodi, P.M.B. 21023, Ikeja, Lagos, Nigeria
*Corresponding author

Abstract

The production of pectinase from two strains of *Aspergillus niger* (A and AA1) were carried out using corn pomace as substrate. The activities of the crude pectinase from *Aspergillus niger* strains A and AA1 were 139100 U/mg and 156740 U/mg respectively. Specific pectinase activities of strains A and AA1 were observed to be 21609.45 U/mg and 3288.22 U/mg respectively. Ammonium sulphate precipitation of pectinase from both strains had their activities increased progressively on 60-80% concentratiion. An optimum temperature and pH of 50 °C and 5.0 respectively were observed for pectinase of both strains. Thermal stability studies showed that the pectinase from both strains of *Aspergillus niger* could withstand heat at 50 °C for 150 minutes. Microbial production of pectinase by strains of *Aspergillus niger* from agro industrial waste is discussed in line with our findings.

Keywords: Pectinase, *Aspergillus nig*er, agro-based waste, crude activity, and ammonium sulphate precipitation.

Introduction

The biotechnological importance of pectinolytic enzymes from microbial source has attracted the attention of many researchers as a result of its numerous applications in various industrial processes. Enzyme that hydrolyzes or degrades pectin (a complex polysaccharide), which is one of the major structural components of plant cell wall, is known as pectinase. These enzymes are one of the most important groups of biological catalysts that have been established for usage in increasing juice yield and clarification in fruits and vegetable industries. Pectinase is used in the treatment and degumming of natural fibers used in paper and textile industry. Pectinase plays a significant role in coffee and tea fermentations. They are used in animal feed production for reducing feed viscosity, increase nutrients absorption, release nutrients by hydrolysis of non-biodegradable fibers or by releasing nutrients blocked by these fibers and reduces the amount of nutrients in faeces.

According to their mode of action, they can be classified into endo-pectatelyase, exo-pectatelyase, exo-poly α-galactouronosidase, galacturan (1, 4) α-galactouronidase, polygalacturonase, pectinesterase, and endopectinylase. It has been well documented, that several organisms are able to produce pectin-degrading enzymes including plants, filamentous fungi, bacteria and yeast (Silva, 2005). For industrial purposes moulds such as *Aspergillus niger*, *Coniotryriumdiplodiela*, *Penicillum* and *Rhizopus species* are preferred because as much as 90% of the enzyme can be secreted into the culture medium. *Aspergillus niger* is used for industrial production of pectinase owing to its GRAS (Generally Regarded As

Safe) status, which allows its product to be used in food-related applications (Naidu and Panda, 1998).
Thermally stable liquid enzymes have drawn a lot of attention as they are associated with many industrial benefits. They are non-polluting, safe and easy to use biochemical catalysts. In the industries, liquid enzymes mix with process medium instantly and distributed evenly as against powdered enzymes. It has been reported that pectinase contributes to more than 25 % of the global enzyme sales (Jayani *et al.,* 2005). In order to meet this high demand, it is highly important to produce pectinase enzyme in a cost effective and productive way. Several agro- wastes including citrus wastes, wheat bran, sugar beet, sugar cane, corn and wheat flour have been utilized for pectinase production by solid state fermentation.

Two types of fermentations can be carried out for the pectinase production. They are solid state fermentation and submerged fermentation. In comparison between these two fermentations, solid state fermentation presents a series of advantages over submerged state fermentation. Culture conditions are similar for the filamentous microorganisms as in the case of solid state fermentation. The growth of organisms is very high with large quantities of enzyme being produced (Ramanujam *et al.,* 2008). This study was done for the purpose of producing pectinase from two strains of *Aspergillus niger* (strain A and AA1) by solid state fermentation using agro industrial waste. Furthermore, partial purification of the crude pectinases was done at various ammonium sulphate concentrations. Efforts were also made to compare the characteristic of pectinases produced from the two strains with respect to thermal stability, optimal pH and temperature.

Materials and Methods

Microorganisms

Two pectinase-producing strains of *Aspergillus niger* designated as A and AA1 were obtained from the culture bank of Federal Institute of Industrial Research, Oshodi (FIIRO) Nigeria. The strains were sub cultured on potato dextrose agar (oxoid).

Screening for pectinolytic activity

The screening methodology involves the use of Pectinase Screening Agar Medium (PSAM) containing: 1 gm pectin; 0.3 gm Disodium hydrogen orthophosphate; 0.2 gm KH_2PO_4; 0.3 gm K_2HPO_4; 0.01 gm $MgSO_4$ and 2.5 gm agar in 100 ml of distilled water. The initial pH of medium was adjusted to 4.5. This medium was sterilized and distributed aseptically in petri dishes. The petri dishes containing PSAM were inoculated with *Aspergillus niger* and incubated at 30 °C for 48 hrs. At the end of the incubation period, the plates were stained with iodine solution as described by Yogesh *et al*., (2009); with some modifications.

Production medium

The fermentation medium which consists of 50 gm corn pomace; 5 gm soy bean powder and pectin powder (2 g) were mixed with 70 ml of mineral water containing 0.124 gm $ZnSO_4.7H_20$; 0.126 gm $FeSO_4.7H_20$ and 0.016 gm $CuSO_4.5H_20$ as substrate. The pH of the medium was adjusted to 4.5. The substrate was sterilized at 121 °C for 80 minutes and allowed to cool under aseptic condition. About 20 ml of sterile distilled water containing 2 drops of tween 80 was used to inoculate the fermentation medium with crude spore each strains of *Aspergillus niger*. These were mixed thoroughly under sterile condition, covered with sterile aluminum foil and incubated at 27 °C for 5 days (Akinola and Ayanleye, 2004).

Pectinase extraction

After 5 days of incubation, the fermentation medium was dissolved in 750 ml of sterile sodium acetate buffer (containing 277 ml of 0.1 N sodium acetate and 473 ml of 0.1 N acetic acid, pH 4.5) for 60 min. The mixture was sieved using sterile muslin cloth. The filtrate was used as crude pectinase in further studies.

Pectinase activity assay

The pectinase activity was measured according to the method described by Miller, 1959, with some modifications. The reaction mixture contained 0.5 ml of crude pectinase, 0.5 ml of 1% pectin prepared in sodium acetate buffer (0.05 M; pH 4.5). The mixture was incubated at 50 °C for 30 min. The reaction was stopped with 1.0 ml dinitrosalycyclic acid solution after which the mixture was boiled for 10 min and cooled. A spectrophotometer preset at 540 nm was used in the determination of pectinase activity, while the reducing sugar release was measured using galactouronic acid as standard.

Determination of protein concentration

Total protein contents of the samples were determined according to the method described by Lowry (Lowry *et al.,* 1951). The absorbance was read at 600nm using UV-visible spectrophotometer**.**

Optimum temperature of pectinase

The optimum temperature of pectinase activity was measured using pectin substrate within a temperature range from 20 to 70 °C. Determination of the residual pectinase activity was done using the method employed for pectinase mentioned earlier (Lia *et al.,* 2008).

Optimum pH of pectinase

The optimum pH of pectinase was studied by incubation of the enzyme with 50 mM of sodium acetate buffers (from pH 3 to 8) in the presence of pectin and then residual activity of pectinase was measured.

Thermal stability of pectinase

Pectinase thermal stability was studied by incubating it in a 50 mM sodium acetate buffer at the temperature of 50 °C. At different intervals of 30 minutes, samples were removed and the residual pectinase activity was recorded.

Results and Discussion

Qualitative screening of *Aspergillus niger* strain A and strain AA1 on PSAM showed zone of pectin hydrolysis of 26 mm and 31 mm respectively (Figure 1 and 2). *Aspergillus niger* AA1 had the widest clear zone of pectin hydrolysis (31 mm) on PSAM against the opaque background which is an indication of the ability to degrade pectin into glucose and galaturonic acid as shown in Figure 1. The activities of the crude pectinase from *Aspergillus niger* strains A and AA1 were 139100 U/mg and 156740 U/mg respectively. Specific activities of pectinase from strain A and AA1 were observed to be 2160.95 U/mg and 3288.02 U/mg respectively (Table 1). Nitinkumar *et al.,* (2010) who worked on pectinase produced by solid state fermentation (SSF) using orange bagasse reported the activity of 64 U/gm which was higher than other natural substrates.

SSF holds tremendous potentials for the production of enzymes. The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix. This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials (Tunga and Tunga, 2003). The crude pectinase from both strains of *Aspergillus niger* were pecipitated using ammonium sulphate at various percentages. The specific activities of pectinase from strain A at 60 %, 80 % and 100 % were 9102.04, 9381.32 and 6642.00 U/mg respectively (Table 1). In addition, the specific activities of pectinase from *Aspergillus niger* strain AA1 at 60 %, 80 %, and 100 % ammonium sulphate precipitation were 7799.81, 7840.23, and 5809.04 U/mg respectively (Table 1).

The decrease in activities of pectinase from the two strains with increase in ammonium sulphate concentration is not surprising as the process precipitates out proteins including enzymes. However the decrease in the activities of pectinase during ammonium sulphate precipitation will never be a call for negligence rather it calls for enzymologist to integrate advanced biological techniques such as salting-out using membrane dialyses bags of appropriate molecular weight. It is presumed that integration of salting out will definitely lead to corresponding increase in specific activities of the pectinase enzyme even as percentage of ammonium sulphate increases from 60 % to 100 %.

Pectinase from *Aspergillus niger* (strain A) had specific activities of 18000, 17000, 22000, 20000, and 15000 U/mg at temperatures of 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C respectively (Figure 3). Pectinase from *Aspergillus niger* (strain AA1) had specific activities of 25,000, 2,000, 28000, 28000, 26000, and 18000 U/mg at temperatures 30 °C, 40 °C, 50 °C, 60 °C, 60 °C, and 70 °C respectively. Thus from this study the optimum temperature for the activity of pectinases from both strains is 50 °C. This is the temperature at which hydrolytic action of the pectinase enzyme could be achieved (Figure 3). Banu *et al.,*

(2010) reported an optimum temperature of 35 °C for pectinase produced from fungi in a solids state fermentation study.

Table 1: Ammonium sulphate precipitation of pectinases

Strain A	Strain A1			Strain AA1		
	Activity (U)	Protein concentration (mg/ml)	Specific activity (U/mg)	Activity (U)	Protein concentration (mg/ml)	Specific activity (U/mg)
Crude	139100	64.37	2160.95	156740	47.67	3288.02
60%	223000	24.50	9102.04	240000	30.77	7799.81
80%	232000	24.73	9381.32	301300	38.43	7840.23
100%	306000	46.07	6642.07	261000	44.93	5809.04

Figure 1: Clear zone of pectin hydrolysis of *Aspergillus niger* strain AA1 on PSAM

Figure 2: Clear zone of pectin hydrolysis of *Aspergillus niger* strain A on PSAM

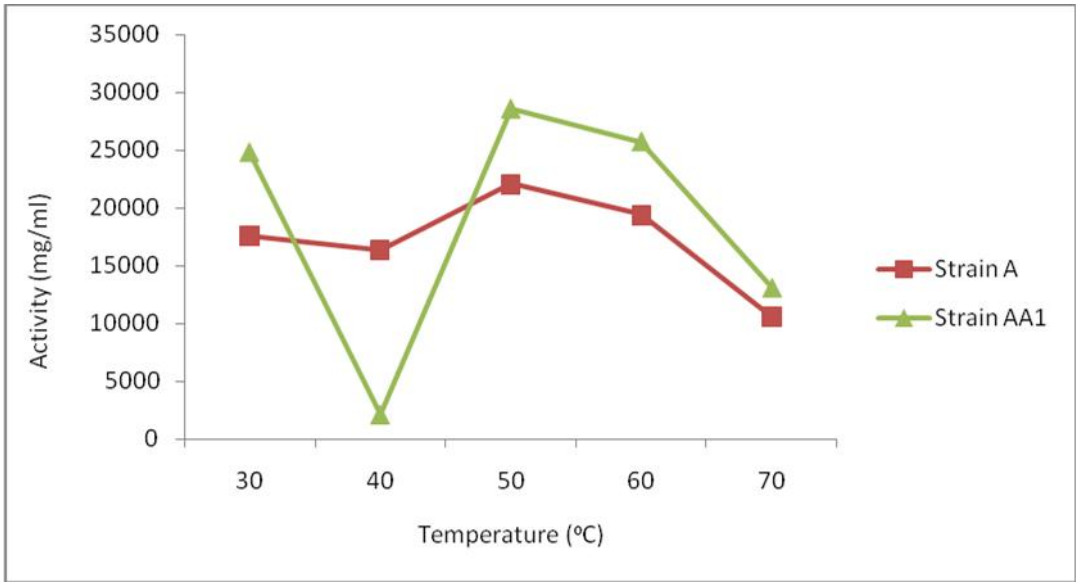

Figure 3: Effect of temperature on the activities of pectinase from two different strains of *Aspergillus niger*

The effect of pH on the specific activities of the two different strains of *Aspergillus niger* was studied. Pectinase of *Aspergillus niger* (strain A) had an activity of 7600 U/mg, 17000 U/mg, 22,000 U/mg, 15,000 U/mg and 13,000 U/mg at pH 3, 4, 5, 6, and 7 respectively (Figure 4). In addition, the specific activity of 15,000, 2000, 33,000, 17,000 and 15,000 U/mg was observed at pH 3, 4, 5, 6, and 7 respectively for pectinase produced from *Aspergillus niger* (strain AA1). Thus the optimum pH for activities of pectinases from the two strains under investigation is 5.0. This is the pH at which pectinase hydrolyses and other applications are best achieved. Rodriguez-Fernandez *et al.*, (2012) studied *Aspergillus niger* pectinase produced by solid state fermentation of dried citrus pulp. The study documented that activity was highest at pH 3.0. Banu *et al.*, (2010) also studied the effectiveness of pH from fungi using related enzyme production medium. Banu *et al.*, (2010) reported an optimum pectinase pH of 6.5. Marcia *et al.*, (1999) studied the stability of polygalaturonase against pH. Their results showed that the pectinase was stable in a pH range of 6-8, but showed highest activity at pH 6.0.

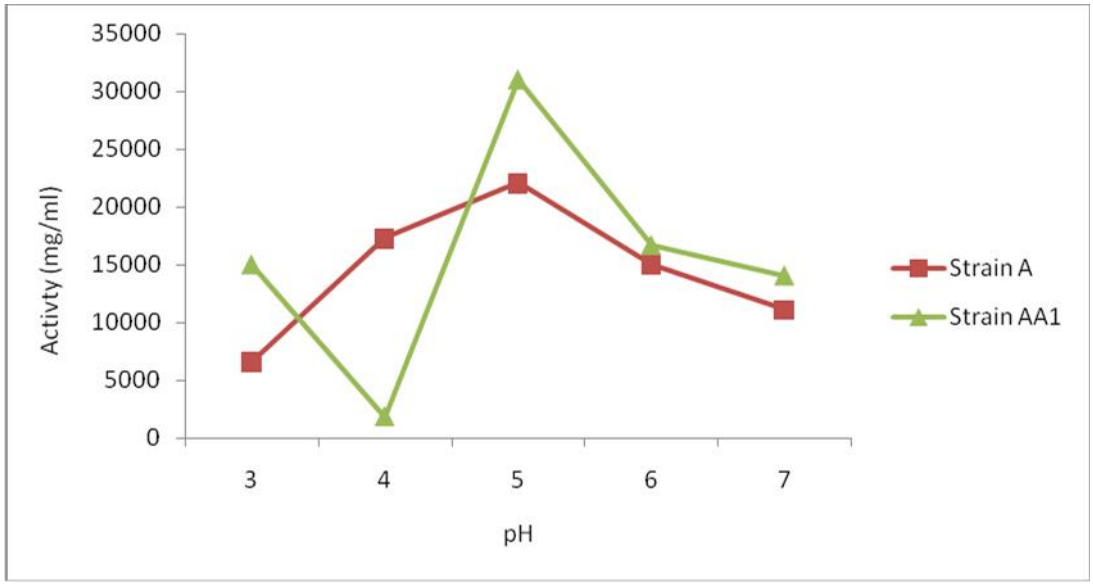

Figure 4: Effect of pH on the activities of pectinase from two different strains of *Aspergillus niger.*

Thermal stability studies showed that the pectinase from both strains of *Aspergillus niger* could withstand heat at 50 °C for 150 minutes. In addition, beyond 150 minutes, activities got reduced significantly from 27,000 U/mg to 22,000 U/mg at 180-210 minutes. However a decrease in activity was only noticed from 150 minutes to 210 minutes for pectinase from strain A (Figure 5). Thus pectinase from strain AA1 is more heat stable than pectinase of strain A. The implication of heat stability is that in industrial processes, such as juice clarification which could proceed at relatively high temperature, catalytic activity can still be successfully achieved. Patil and Chaudri (2010) studied pectinase from fungi using solid state fermentation of agro-based waste. The study reported a temperature of 35 °C as the optimum temperature for the activity of pectinase. Patil and Chaudri (2010) reported that the pectinase is stable at 35 °C for 72 hours. In addition, beyond 72 hours, the activity of pectinase dropped significantly. Martin *et al.*, (2004) reported the production of microbial pectinase which maintained 70 % of its initial stability at 70 °C.

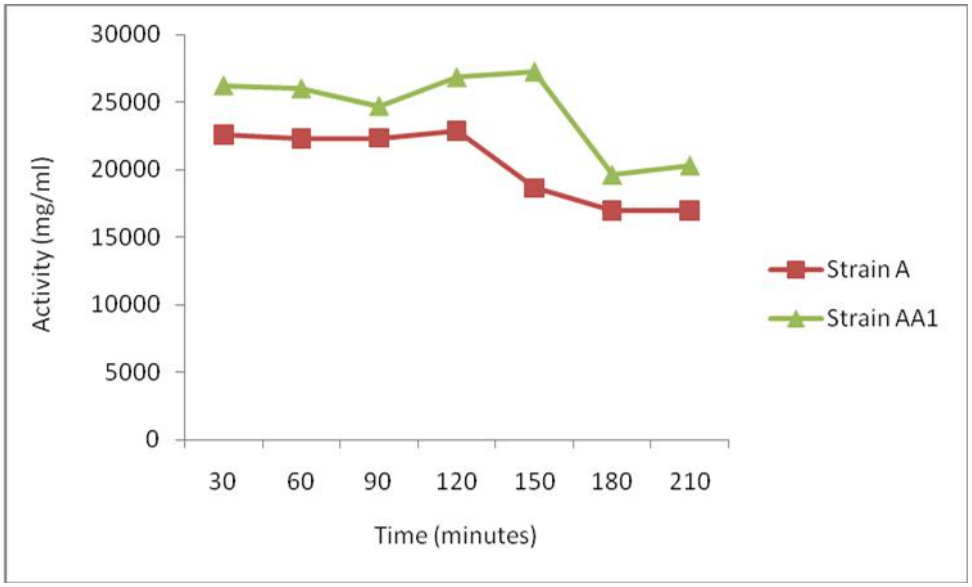

Figure 5: Thermal stability of the pectinase enzyme at 50°C

Conclusion

Several attempts have been put in place all over the world to reduce the cost of enzyme production. Utilization of local raw materials and local production are still the two major factors that influence enzyme cost. In addition, the developed parts of the world have through this approach reduced cost of enzymes. The reverse is still the case for developing and under-developed countries of the world. The case of Nigeria is still worrisome as there are abundant raw materials, and skilled manpower for advances in enzyme technology, yet commercialization of Industrial enzymes is still not feasible. The situation is giving new hope as this study has been able to establish the technology for production of pectinase using corn pomace, an agro-based waste which is readily available and abundant in Nigeria.

References

Akinola, S. O., and Ayanleye, T. A. (2004), The use of fungal glucoamylase enzyme for the production of glucose syrup from cassava starch. *Acta Satech.*, 1(2): 138-141

Babu, A. R., Devi, M. K., Gnanaprabhal, G. R., Pradeep, B. V., and Palaniswamy, M. (2010), Production and characterization of pectinase enzyme from *Penicillium chrysogenum, Indian Journal of Science and Technology*, 3(4): 377-381

Jayani, R. S., Saxena, S., Gupta, R. (2005), Microbial pectinolytic enzymes: A review. *Process Biochem*., 40(9): 2931-2944

Lia, T., Lib, S., Wanga, N. and Taina, L. (2008), Immobilization and stabilization of pectinase by multipoint attachment onto an activated agar-gel support. J. Food Chem. **109**:703-708.

Lowry, O. H., Rosebrough, N. J., Farr A. L., and Randall, R. J., (1951), Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193, 265-275.

Martin, N., Souza, S. R, Silva, R. and Gomes, E., (2004), Pectinase production by fungal strains in solid state fermentation using agro-industrial bio product. *Braz. Arch. Biol. Technol. 47:* 813-819.

Miller, G. L. (1959), Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*., 31: 426-428.

Naidu, G. S. N., and Panda, T. (1998), Application of response surface methodology to evaluate some aspects on stability of pectolytic enzymes from *Aspergillus nige*r. *Biochem.Eng*. p. 71-77.

Neeta, R. S., Anupama, S., Anjuvan, S., and Giridhar, S. (2011), Production of polygalaturonase and pectin methyl esterase from agro waste by using various isolates of *Aspergillus niger*. *Insight Microbiology 1 (1): 1-7.*

Nitinkumar, P., Patil and Bhushan L. Chaudhari, (2010), Production and purification of pectinase by soil isolate *Penicillum sp* and search for better agro-residue for its SSF. *Recent Research in Science and Technology*, 2(7): 36-42.

Ramanujam, N., Saritha, Palanisubramani. (2008), Production of pectiniyase by solid-state fermentation of sugarcane bagasse using *Aspergillus niger .Advanced Biotech*:30-33.

Silva, E. G., Borges, M. F., Medina, C., Piccoli, R. H., Schwan, R. F. (2005), Pectinolytic enzymes secreted by yeast from tropical fruits.*"FEMS Yeast Research.*Vol. 5, p. 859-865.

Rodríguez Fernández D. E, Rodríguez León J. A, de Carvalho J. C, Karp S. G, Parada J. L, (2012), Process development to recover pectinases produced by Solid-State Fermentation. *J Bioprocess Biotechniq*., 2(121): 1-7

Patil, N. P., and Chaudhari, B. L., (2010), Production and purification of pectinase by soil isolate *Penicillium* sp and search for better agro-residue for its SSF, *Recent Research in Science and Technology, 2(7): 36-42*

Tunga, R., and Tunga, B. S. (2003), Extracellular amylase production by *Aspergillus oryzae* under solid state fermentation. *International centre for Biochemistry*, Olaka University, Japan; 12.

Yogesh K., Vamsi K. K., Amol B., Nikhil G., Soham T., Prasad P., Girish G., Mayank G., Amol J., Adarsh M., Joshi B., and Mishra D , (2009), Study of pectinase production in submerged fermentation using different strains of *Aspergillus niger. International Journal of Microbiology Research, ISSN: 0975-5276, Volume 1, Issue 2, pp-13-17.*