Trop J Nat Prod Res, October 2021; 5(10):1876-1882 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Enhanced Production, Purification and Characterization of Pectinase from *Aspergillus flavus*

Chery I. Nwokeoma[1,2]*, Daniel J. Arotupin[2], Oladipo O. Olaniyi[2], Fatusa C. Adetuyi[2]

[1]*Department of Microbiology, Federal University, Oye-Ekiti, Ekiti state, Nigeria*
[2]*Department of Microbiology, Federal University of Technology, Akure, Ondo state, Nigeria*

ARTICLE INFO

Article history:
Received 10 September 2021
Revised 13 October 2021
Accepted 21 October 2021
Published online 02 November 2021

Copyright: © 2021 Nwokeoma *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Pectinase is a crucial enzyme in the food, drink and biotechnology industry, whose market is projected to speedily advance within the next few years. The aim of this study was to enhance the production of pectinase from previously isolated *Aspergillus flavus*, to purify the pectinase and to characterize the purified pectinase. This study was designed based on the one-factor-at-a-time approach and submerged state fermentation using rice husk as carbon source was employed. Conditions that favored pectinase production were an incubation temperature of 50°C, pH of 4.5 and the addition of 1% maltose and yeast extract as carbon and nitrogen sources. The pure enzyme had its optimum activity at 40^{0}C and at pH 6. It also had its peak activity with 5 mM mercury II chloride but was inhibited by the presence of tin II chloride. The purified pectinase had a K_m value of 3.33 mg/mL and V_{max} of 16.98 U/min. These findings demonstrate that *A. flavus* produced pectinase with attributes that could be useful in biotechnological industries.

***Keywords*:** *Aspergillus flavus*, Pectinase, Production, Purification, Rice husk.

Introduction

Pectins are generally known as a family of complex polysaccharides, primarily composed of α-1→4 linked D-galacturonic acid residues. They have a small number of rhamnose residues in the core chain along with arabinose, xylose and galactose on their side chains and are found more at the middle lamella in cell walls and intercellular tissues of plants, where they contribute to the firmness and structural rigidity of vegetal tissues. Constituting one-third of total sugars in plant materials, they appear as magnesium and calcium pectate.[1,2] Pectinases are enzymes that effectively de-esterify and depolymerize pectic substances by transelimination. These pectinases usually degrade and modify pectin to yield galacturonic acid, rhamnose and smaller portions of simpler sugars like arabinose, acetate, galactose, fructose and xylose, which microbes then metabolize and assimilate for their growth and possibly, for survival.[3,4] Fungal pectinases are mostly preferred to bacterial pectinases because they are produced in large quantities extracellularly and can be recovered with relative ease.[24,40] Fungi produce pectinases to aid in the breakdown of the middle lamella in plants to enable them extract nutrients from the plant tissues and insert their hyphae. The pectinase produced foment biological deterioration of organic matter by modifying pectin. Pectinases are reported to be the first set of enzymes secreted by fungi prior to subsequent degradation by other enzymes.[5,6,7] Pectinase is secreted by many fungi, but all microorganisms utilized for commercial enzymes must be those classified as GRAS- generally regarded as safe.[24] A lot of usefulness is also attributed to pectinases' ability to convert pectin, and these have been exploited for the benefit of man especially in industries. In the drink industries, they aid juice extraction, cloud stabilization, and reduction of wine harshness. In the food industry, they aid in macerating fruits, in the refinement of vegetable fibers, in baby foods manufacture, for oil extraction, for coffee and tea seed fermentation and foam prevention. They are also extensively employed in textile, pharmaceutical, waste-water treatment, detergent-making, pulp and paper-producing and wood-processing industries, among others.[8-12] Medically, pectinases have been found to act as antitumor agents. They can stimulate apoptosis in human colonic adenocarcinoma and in melanoma cells and reduce proliferation of myeloma cells.[13,14]
Purification and characterization of pectinase in this study is important because the intrinsic features peculiar to the enzyme, such as favourable functioning conditions, can only be determined when it has been separated from all other compounds that may activate or inhibit its functions. The aim of this study was to determine the optimum conditions for pectinase production by *A. flavus*, to purify the pectinase and to characterize the purified pectinase.

Materials and Methods

Fungus used as inoculum
Aspergillus flavus that had been identified by molecular method, was the inoculum used in this study. It had been screened from rice husk mill in Ire-Ekiti, using Pectinase isolation media and identified as the best pectinase producer, where it gave the highest activity among other isolates, in a separate (unpublished) study on pectinase carried out in the Research Laboratory of the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. It was maintained on Potato Dextrose agar at 4°C.

Pectinase production in submerged fermentation
Pectinase production was carried out based on the method of Thangaratham and Manimegalai.[16] Two hundred and fifty milliliters (250 mL) conical flasks, each having 150 mL production media, contained the following in gL^{-1}: 10 g rice husk, 6.0 g K_2HPO_4, 6.0 g KH_2PO_4, 6.0 g NH_4SO_4 and 0.1 g $MgSO_4.7H_2O$ (adjusted to a pH of 5.4±0.2 using 1M HCl or 1M NaOH).[16] Sterile media were inoculated with 1 mL of 10^5 spores mL^{-1} derived from 5-day old slant on PDA

*Corresponding author. E mail: cherylnwokeoma@yahoo.com; cherylnwokeoma@gmail.com
Tel: +234 9038768762

Citation: Nwokeoma CI, Arotupin DJ, Olaniyi OO, Adetuyi FC. Enhanced Production, Purification and Characterization of Pectinase from *Aspergillus flavus*. Trop J Nat Prod Res. 2021; 5(10):1876-1882. doi.org/10.26538/tjnpr/v5i10.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

into which 10 mL distilled water had been dispersed. The samples were then incubated under shaking conditions for 72 h at 28±3°C and 120 rpm. At the interval of 24 hours, samples were withdrawn for enzyme assay.

Assay for pectinase in crude supernatant
One milliliter of supernatant from the centrifuged fermentation broth, was mixed with 1 mL of 2% pectin solution (citrus pectin from Sigma-Aldrich, dissolved in 0.1 M acetate buffer, pH 5.0) and incubated at 40^0C for 10 minutes. Following Miller's method of determination of reducing sugar,[47] the sample was boiled for 5 minutes after addition of 3 mL 3,5-dinitrosalicylic acid (DNSA). After cooling on ice, its absorbance was read at 540 nm with 752Pro UV-VIS spectrophotometer (Spectrum Lab, England). One unit of pectinase activity (U) was defined as the amount of enzyme which liberated 1 µmol galacturonic acid per minute under standard assay conditions. Standard curve was prepared using D-galacturonic acid.

Optimization of pectinase production
Effect of incubation temperature on enzyme production
Samples were incubated under shaking conditions of 120 rpm, at temperatures of 20, 30, 40 and 50 ^{0}C for 72 h, and aliquot samples were withdrawn every 24 h for enzyme assay.

Effect of pH on enzyme production
pH of the fermentation media was varied using pH values of 4.0, 4.5, 5.0, 5.5, 6.0. These were incubated under shaking conditions (120 rpm) at a temperature of 30^0C for 72 h, and aliquot samples were withdrawn every 24 hours for enzyme assay.

Effect of carbon source on enzyme production
Different carbon sources; fructose, galactose, lactose, maltose, sucrose, and starch, were added in place of rice husk, at the rate of 10 gL^{-1}into fermentation broth. The broth was sterilized, inoculated and incubated for 72 h at 120 rpm, and aliquot samples were withdrawn every 24 h for enzyme assay.

Effect of nitrogen source on enzyme production
Sodium nitrate ($NaNO_3$), ammonium nitrate (NH_4NO_3), Yeast extract, Tryptone and Urea were each utilized as nitrogen sources in place of NH_4SO_4, at the rate of 10 gL^{-1}. The fermentation broth was then sterilized, inoculated and incubated under shaking conditions, for 72 h, and aliquot samples were withdrawn every 24 h for enzyme assay.

Determination of fungal biomass weight
The growth of the fungus was examined to determine if enzyme production increased or decreased with growth of fungi (deduced by its biomass). The fungus' biomass was calculated using Arotupin and Ogunmolu's method.[17] At the end of fermentation, biomass of fungus was obtained daily by oven drying at 60^0C, until constant weights were obtained. These were weighed to obtain dry weight of biomass. Biomass was calculated thus:

$$W = Fr - Fi \quad [1]$$

where W = weight of fungi measured in g/L
Fr = weight of residue on filter paper
Fi = initial weight of filter paper

Purification of pectinase
The crude enzyme was subjected to 80% ammonium sulfate saturation and left at 4°C for 12 hours. Precipitate recovered was then dissolved in 0.1 M citrate buffer (pH 5.0) and dialyzed with the buffer to remove ammonium sulfate. Sephadex C-50 was loaded with 5 mL dialyzed enzyme and then collected in fractions at the rate of 5 mL per 20 minutes. The column was first washed with the buffer before being eluted by 0.5 M and then 1.0 M sodium chloride in buffer. Pectinase activity and protein concentration were monitored using spectrometer read at 540 and 280 nm respectively.

Protein content determination
Protein content determination in enzyme solution was done according to Lowry *et al*.[18]. Bovine serum albumen (BSA) was used as standard. Absorbance at 590 nm using 752Pro UV-VIS spectrophotometer (Spectrum Lab, England) were read and recorded.

Characterization of purified pectinase
Effect of temperature on purified pectinase
The method of Sulyman *et al*. [19] was employed. The incubation temperature was varied from 30-60°C for the mix of 0.1 mL purified pectinase, in 0.5 mL of 1% pectin. The effects of these were determined by assaying for enzyme activity.

Effect of pH
Buffers of different pH (citrate buffer: 3.0–5.0, phosphate buffer: 6.0–8.0, borate buffer 9.0–11.0) were used to make 1% pectin solution. A volume of 0.5 mL of this pectin solution was mixed with 0.1 mL of purified pectinase. These were incubated for 10 mins at 40°C prior enzyme assay.

Effect of salts and other chemical agents
The effects of different salts, mercury II chloride ($HgCl_2$), zinc chloride ($ZnCl_2$), tin II chloride ($SnCl_2$), potassium chloride (KCl); and three chemicals; ethylenediaminetetraacetic acid (EDTA), urea and 2-mercaptoethanol, on pectinase activity were assessed. The salts and chemicals were utilized at concentrations of 1, 5 and 10 mM and solubilized in distilled water. The enzyme was pre-incubated for 10 minutes at 40°C with the salts and chemicals prior to the addition of pectin and assay for enzyme activity. Assay mixtures for each group without the salts or chemicals served as controls.

Determination of kinetic parameters
The V_{max} and K_m of pectinase was determined for citrus pectin using concentrations of 1.25–10.00 mg/mL. The data obtained were used to plot Lineweaver and Burk graph.[44]

Statistical analysis
The results presented are means of triplicate readings. One-Way Analysis of Variance (ANOVA) with SPSS version 20.0 was used to analyze data and the results were subjected to Duncan's new multiple range test at 95% confidence level.

Results and Discussion

This study presents rice husk as a good source of carbon for fungal growth and pectinase production, even though husk is known to contain modest amounts of pectin.[15] *Aspergillus* spp have been extensively studied for pectinase production as seen in various studies.[20-24]

Effect of optimization parameters on pectinase production
The best pectinase activity was recorded at a temperature of 50°C at 24 hours (Figure 1a). Similar results were also observed in separate studies by researchers in pectinase production using sucrose[25, 26] as carbon source. Phutela *et al*.[25] and Rubinder *et al*.[27] reported that some fungi produced enzymes optimally at 50ºC, like the result obtained in this study. As inferred by Khatri *et al*.,[37] this result indicates that of the fungus is a thermophile, since it could produce active enzyme at that temperature. The pH 4.5 promoted the highest pectinase activity (Figure 1b). This result was similar to that observed in another study using rice bran for pectinase production.[16] Phutela *et al*. [25] had observed that slightly acidic pH ranges of 4.0-5.0 promoted high polygalacturonic acid and pectinase production, probably because these fungi, like fungi in general, thrive best in slightly acidic environments. Refined saccharides were utilized for the carbon source trial. Maltose was observed to be the best pectinase promoter (Figure 1c) among all carbon sources investigated, similar to the report in another study.[17] Although all other carbon sources in this study (see Figure 1c) promoted a good activity of pectinase, fructose, gave the least pectinase activity each day, significantly lower ($P \leq 0.05$) than other carbon sources. This result was contrary to the findings of Ire and Viking,[29] where fructose gave the highest pectinase production.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

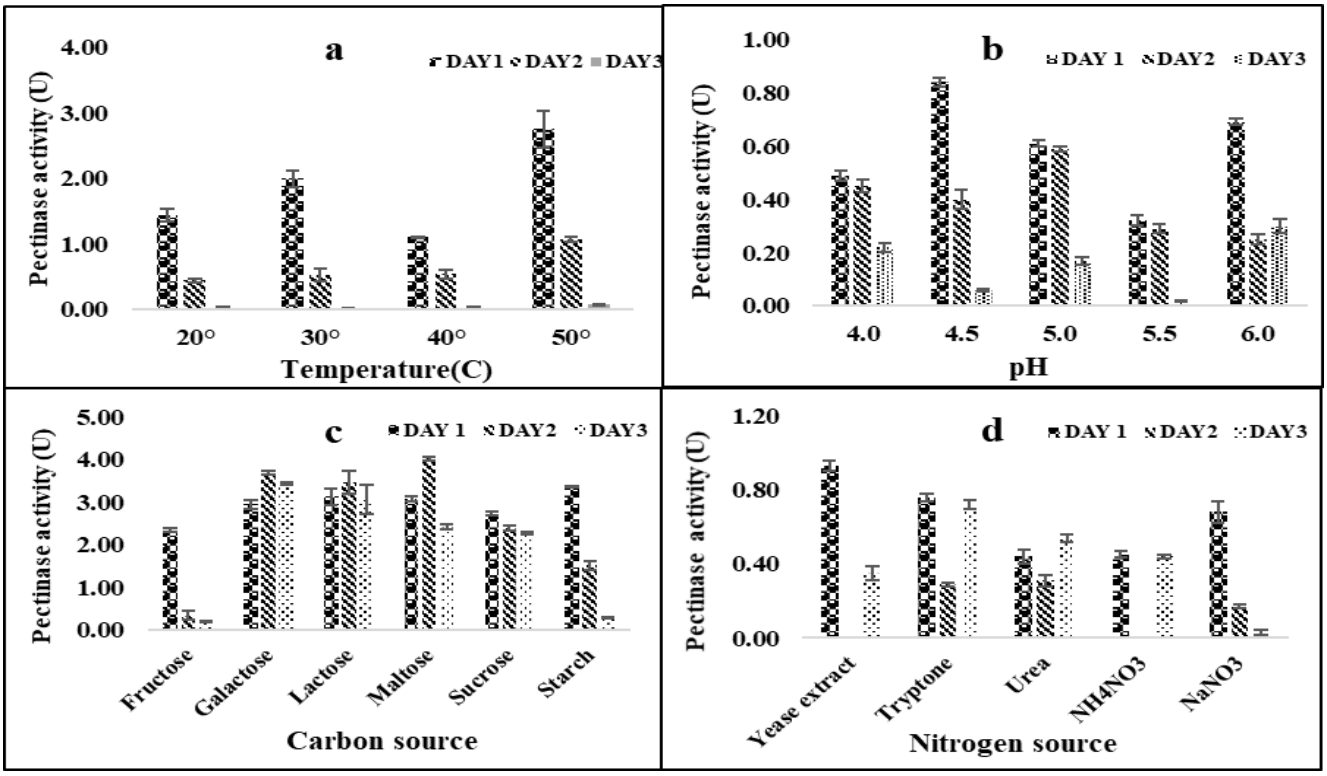

Figure 1: Optimization of (a)incubation temperature; (b) pH; (c) carbon sources; (d) nitrogen sources on pectinase production by *A. flavus* for three days.

These different reactions to carbon may be caused by non-assimilation of the carbon into cells, a lack of the metabolic pathway to metabolize the carbon or the possibility that the carbon is hazardous to the cells.[39] Yeast extract induced the highest pectinase activity (0.93±0.027U), when different nitrogen sources were investigated (Figure 1d). Yeast extract was documented by some researchers as the best nitrogen source for pectinase production using *Bacillus subtillis*,[30] and *Aspergillus* spp, [28,31] respectively. Yeast extract, like other organic nitrogen sources, have been known to promote fungal metabolism and enzyme production due to their content of nutritional and growth factors like vitamins.[28]

Effect of investigated parameters on fungal biomass

In this study, the highest biomasses were observed at the temperature of 50 ^{0}C and pH 5.0 on the third day (Figure 2a and b) while, that for carbon and nitrogen sources were obtained with sucrose on day 1 and urea on day 3 (Figure 2c and d) respectively. Although maltose-enriched media yielded the best pectinase value overall, growth were best with the sucrose-enriched media at 24 hours. This may be because the fungi did not assimilate the carbon well or because it lacked a pathway to properly metabolize this carbon.[39] There was regression in growth from day 1 through day 3 for all treatments in the carbon source trials except for lactose and maltose. This decrease in growth may be due to a reduction in readily available carbon necessary for metabolism.[38] In general, the growth of the fungi (represented by biomass weight) was not seen to affect its enzyme production rate.

Summary of purification of pectinase

The crude pectinase had an initial total activity of 225.2 U while its initial specific activity was 0.61 U/mg (Table 1). Ammonium sulfate precipitation lowered the total activity to 150.1 U while increasing the specific activity to 1.14 U/mg, with a 35.5% yield. At the final stage of purification, using ion exchange chromatography, total activity reduced to 62.7 U, with a total protein of 48.1 mg while specific activity rose slightly to 1.30 U/mg. Purification ended with a 12.9% yield and a 2.13 purification fold. This low yield could be as a result of the loss of some activity during the purification process.[46]

Characterization of the pure pectinase

The purified pectinase had an optimum temperature of 40°C (Figure 3a), similar to some reports.[29,32] *Aspergillus* spp have been known to produce fairly high-temperature acting enzymes.[34] A pH of 6.0 was shown to produce the highest relative activity (1.24 %) for the purified enzyme (Figure 3b). Reports show that there is usually high pectinase activities around pH 3.5 to about pH 9.[34,35] The ability of salts to enhance the activity of purified pectinase was shown in Table 2. All the salts tested in this study produced a 20% lesser activity than 5 mM $HgCl_2$. In this study, urea was found to promote enzyme relative activity with concentration of 1 mM as opposed to the study where urea was one of the least promoters of pectinase activity.[17] An increase in EDTA concentration yielded improved activity from 5 mM concentration as supported by other studies.[41,42] These results display the potential of this pectinase to still perform well in the presence of 1-10 mM $HgCl_2$ and 5-10 mM EDTA.

Kinetic parameters of purified pectinase from A. flavus

A Michaelis Menten's constant, K_m, of 3.33 mg/mL was obtained from Lineweaver-Burk plot of pectinase (Figure 4), showing the effect of varying concentrations of citrus pectin (1.25 mg/mL – 10 mg/mL). Close values of 3.08 mg/mL and 3.2mg/mL were obtained with *A. fumigatus*[24] and *A. tubingensis.*[35] A considerable number of pectinases have been shown to have K_m values between 0.1 and 5.0,[34] like the value in this study. A maximum velocity, V_{max}, of 16.98 U/mL obtained in this study, was similar to the 16 U/mg obtained[37] from *Fusarium oxysporum f.* sp. *vasinfectum* using polygalacturonic acid as substrate. The maximum velocity represents the point of maximum catalysis during an enzyme-catalyzed reaction at the instance of substrate saturation. Low values of K_m like that obtained in this study, reflect affinity of enzyme to substrate, while higher values mean otherwise.[43]

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

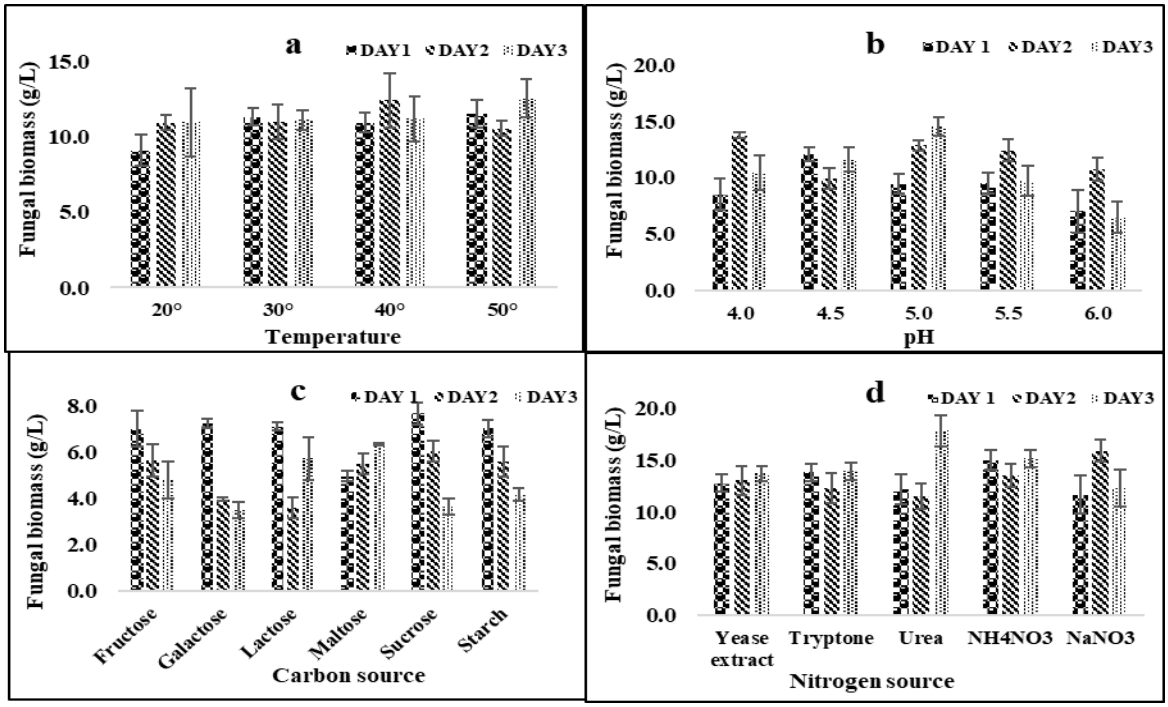

Figure 2: Optimization of (a) incubation temperature; (b) pH; (c) carbon sources; (d) nitrogen sources; on biomass of *A. flavus* on for three days.

Table 1: Summary of purification of pectinase

Purification steps	Total Activity (U)	Total Protein (mg/mL)	Specific activity (U/mg)	Yield %	Purification Fold
Crude enzyme	225.2	371.7	0.61	100	1.00
Ammonium sulfate precipitate (80 %)	150.1	131.8	1.14	35.5	1.87
Ion-exchange chromatography	62.7	48.1	1.30	12.9	2.13

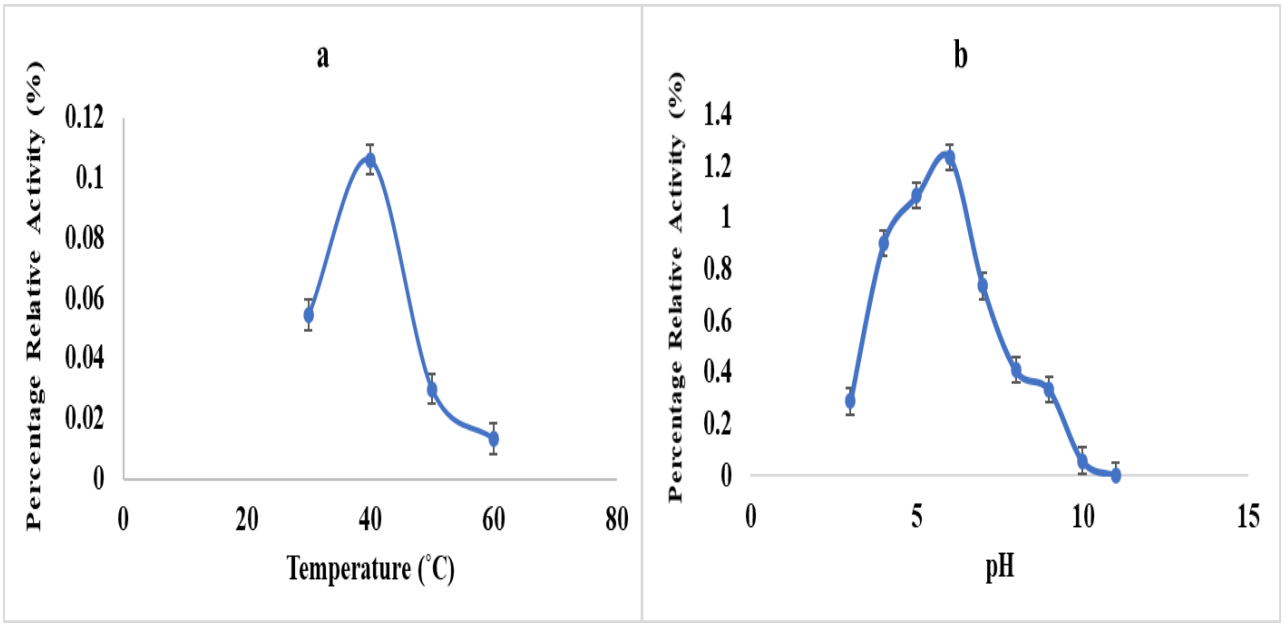

Figure 3: Effect of (a) temperature; (b) pH; on the activity of purified pectinase

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Influence of salts and chemicals on the activity of the purified pectinase

Concentration	% Residual activity		
	1.00 mM	5.00 mM	10.00 mM
Control	100.00	100.00	100.00
Salts			
$HgCl_2$	130.80	142.30	106.70
$SnCl_2$	100.10	90.00	90.00
$ZnCl_2$	113.00	92.90	90.00
KCl	99.90	90.00	111.80
Chemicals			
EDTA	99.98	120.70	129.10
Urea	112.70	90.00	90.00
2-Mercaptoethanol	99.90	90.00	123.30

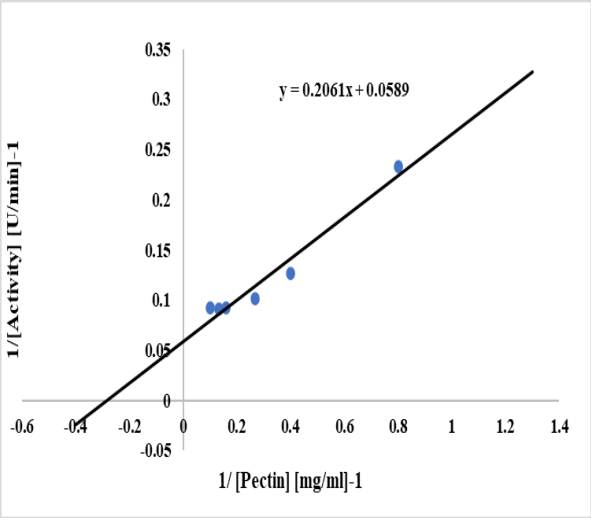

Figure 4: Lineweaver-Burk plot of pectinase showing the effect of varying concentration of citrus pectin on pectinase from *Aspergillus flavus*. K_m = 3.33 mg/mL V_{max} = 16.98 U/min

Conclusion

This study was done to enhance the production of pectinase by *A. flavus*, to purify the pectinase and to characterize the purified pectinase. The production of pectinase was enhanced by a temperature of 50°C, pH of 4.5 and the presence of maltose and yeast extract as carbon and nitrogen sources, respectively. The purified pectinase had a yield of 12.9%. The characterized pure pectinase had its highest activity at temperature of 40°C, pH of 6.0 and in the presence of $HgCl_2$, Urea and EDTA. These findings demonstrated that the purified pectinase possessed attributes that are desirable in lignocellulose-utilizing and biotechnological industries where pectinases find application.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Trindade LV, Desagiacomo C, Polizeli ML, Damasio AR, Lima AM, Gomes E, Bonilla-Rodriguez GO. Biochemical Characterization, Thermal Stability, and Partial Sequence of a Novel Exo-Polygalacturonase from the Thermophilic Fungus *Rhizomucor pusillus* A13.36 obtained by Submerged cultivation. Biomed Res Int 2016; 2016, Article ID 8653583.
2. Kameshwar SAK and Qin W. Structural and functional properties of pectin and lignin–carbohydrate complexes de-esterases: a review. Bioresour Bioprocess 2018; 5: Article number 43(2018).
3. Prade RA, Zhan D, Ayoubi P, Mort AJ. Pectins, Pectinases and Plant-Microbe Interactions. Biotechnol Genet Eng Rev. 1999; 16(1):361-391
4. Caffall KH and Mohnen D. The structure, function and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr Res. 2009; 344(14):1879-1900.
5. Mrudula S and Anitharaj R. Pectinase production in solid-state fermentation by *Aspergillus niger* using orange peel as substrate. Glob J Biotech Biochem. 2011; 6(2):64-71.
6. Jayani RS, Saxena S, Gupta R. Microbial pectinolytic enzymes: A review. Process Biochem. 2005; 40(2005):2931-2944.
7. De Lorenzo G, D'Ovidio R, Cervone F. The role of polygalacturonase-inhibiting proteins (PGIPs) in defense against pathogenic fungi. Annu Rev Phytopathol. 2001; 39(1):313-335.
8. Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R. Microbial pectinases: an ecofriendly tool of nature for industries. Biotech. 2016; 6(1):47.
9. Satapathy S, Rout JR, Kerry RG, Thatoi H, Sahoo SL. Biochemical Prospects of Various Microbial Pectinase and Pectin: An Approachable Concept in Pharmaceutical Bioprocessing. Front Nutr. 2020; 7:Article 117.
10. Nighorjkar A, Patidar MK, Nighorjkar S. Pectinases: Production and Applications for Fruit Juice Beverages. In Processing and Sustainability of Beverages. Digital Science Inc. 2019; 235-273 p.
11. Bhardwaj V, Degrassi G, Bhardwaj RK. Microbial Pectinases and their applications in industries: A review. Int Res J Eng Technol. 2017; 04(08):829-836
12. Patidar MK, Nighojkar S, Kumar A, Nighojkar A. Pectinolytic enzymes‑solid state fermentation, assay methods and applications in fruit juice industries: a review. 3 Biotech. 2018; 8(4):199.
13. Vayssade M, Sengkhamparn N, Verhoef R, Delaigue C, Goundiam O, Vigneron P, Voragen, AG, Schols HA, Nagel MD. Antiproliferative and proapoptotic actions of okra pectin on B16F10 melanoma cells. Phytother Res. 2010; 24(7):982-989.
14. Leclere L, Van Cutsem P, Michiels C. Anti-cancer activities of pH- or heat-modified pectin. Front Pharmacol. 2013; 4: Article 128
15. Khandanlou R, Ngoh GC, Chong WT. Feasibility study and structural analysis of cellulose isolated from rice husk: Microwave irradiation, optimization, and

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

treatment process scheme. BioResour. 2016; 11(3):5751-5766.
16. Thangaratham T and Manimegalai G. Optimization and production of pectinase using agro-waste by solid state and submerged fermentation. Int J Curr Microbiol Appl Sci. 2014; 3(9):357-365.
17. Arotupin DJ and Ogunmolu FE. Experimental Investigations on the Effects of Carbon and Nitrogen Sources on concomitant amylase and polygalacturonase Production by *Trichoderma viride* BITRS-1001 in submerged fermentation. Biotechnol Res Int. 2012; 2012: Article ID 904763
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-275.
19. Sulyman AO, Igunnu A, Malomo SO. Isolation, purification, and characterization of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells. Heliyon 2020; 6(12):e05668.
20. Blandino A, Iqbalsyah T, Pandiella S, Cantero D, Webb C. Polygalacturonase production by *Aspergillus awamori* on wheat in solid-state fermentation. Appl Microbiol Biotechnol. 2002; 58(2):164.
21. Al-Rajhi AM. Purification and Characterization of an Extracellular Poly-galacturonase from *Rhizoctonia solani Kühn* (AG2-2). World Appl Sci J. 2013; 21(4):476-484.
22. Sethi BK, Nanda PK, Sahoo S. Enhanced production of pectinase by *Aspergillus terreus* NCFT 4269.10 using banana peels as substrate. 3 Biotech. 2016; 6(1):36.
23. Ketipally R and Ram MR. Optimization of Pectinase Production by *Aspergillus oryzae* RR 103. Curr Agric Res J. 2018; 6(1):37-44.
24. Okonji RE, Itakorede BO, Ovumedia JO, Adedeji OS. Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials. J Appl Biol Biotechnol. 2019; 7(03):1-8.
25. Phutela U, Dhuna V, Sandhu S, Chadha BS. Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposing orange peels. Braz J Microbiol. 2005; 36(1):63-69.
26. Bhardwaj V and Neelam G. Production, Purification of Pectinase from *Bacillus* sp. MBRL576 isolate and its application in extraction of juice. Int J Sci Res. 2014; 3(6):648-652.
27. Rubinder K, Chadha BS, Singh N, Saini, HS, Singh S. Amylase hyper production by de-regulated mutants of the thermophilic fungus *Thermomyces lanuginosus*. J Ind Microbiol Biotechnol. 2002; 29(2):70-74.
28. Abdullah R, Farooq I, Kaleem A, Iqtedar M, Iftikhar T. Pectinase Production from *Aspergillus niger* IBT-7 using solid state fermentation. Bangladesh J Bot. 2018; 47(3):473-478
29. Ire FS and Vinking EG. Production, purification and characterization of polygalacturonase from *Aspergillus niger* in solid state and submerged fermentation using banana peels. J Adv Biol Biotechnol. 2016; 10(1):1-15.
30. Kaur SJ and Gupta VK. Production of pectinolytic enzymes pectinase and pectin lyase by *Bacillus subtilis* SAV-21 in solid state fermentation. Ann Microbiol. 2017; 4(67):333-342.
31. Aguilar G, Trejo BA, Garcia JM, Huitron C. Influence of pH on endo and exo-pectinase production by *Aspergillus* species CH-Y-1043. Can J Microbiol. 1991; 37(12):912-917.
32. Ezugwu AL, Ezike TC, Ibeawuchi AN, Nsude CA, Udenwobele DI, Eze SOO, Anyawu, CU, Chilaka FC. Comparative Studies on pectinases obtained from *Aspergillus fumigatus* and *Aspergillus niger* in submerged fermentation system using pectin extracted from mango, orange and pineapple peels as carbon sources. Nig J Biotechnol. 2014; 28(2014):26-34.
33. Sudeep KC, Upadhyaya J, Joshi DR, Lekhak B, Chaudhary DK, Pant BR, Bajgai TR, Dhital R, Khanal S, Koirala N, Raghavan V. Production, Characterization, and Industrial application of pectinase enzyme isolated from fungal strains. Ferment. 2020; 6(2):59.
34. Almulaiky YQ, Albishri AA, Khalil NM, Algamal Y, Aldhahri M, Al-Harbi SA, Al-Talhi HA, Alhadi R. Polygalacturonase by *Aspergillus niger* using seaweed waste under submerged fermentation: production, purification and characterization. Biomed J Sci Technol Res. 2020; 25(4):19416-19422.
35. Kester HCM, Someren KV, Muller Y, Visser J. Primary structure and characterization of an exopolygalacturonase from *Aspergillus tubingensis*. Eur J Biochem. 1996; 240(3):238-246.
36. Liu N, Ma X, Sun Y, Hou Y, Zhang X, Li F. Necrotizing activity of *Verticillium dahliae* and *Fusarium oxysporum f.* sp. *vasinfectum* endopolygalacturonases in cotton. Plant Dis. 2017; 101(7):1128-1138.
37. Khatri BP, Bhattarai T, Shrestha S, Maharjan J. Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolated from Manaslu Conservation Area, Gorkha, Nepal. SpringerPlus. 2015; 4(1):488.
38. Zhou W, Lu Q, Han P, Li J. Microalgae Cultivation and Photobioreactor Design. In Microalgae Cultivation for Biofuel Production. Elservier Inc. 2020:31-50p.
39. Nev OA, Lindsay RJ, Jepson A, Butt L, Beardmore RE, Gudelj I. Predicting microbial growth dynamics in response to nutrient availability. PLoS Comput Biol. 2021; 17(3):e1008817.
40. Souza PM, Bittencourt ML, Caprara CC, Freitas M, Almeida RP, Silvera D, Foneseca YM, Filho EX, Junior AP, Magalhaes PO. A Biotechnology perspective of fungal proteases. Braz J Microbiol. 2015; 46(2):337-346.
41. Jalil MT and Ibrahim D. Partial Purification and Characterization of Pectinase Produced by *Aspergillus niger* LFP-1 grown on Pomelo Peels as a substrate. Trop Life Sci Res. 2021; 32(1):1-22.
42. Losonczi A, Csiszár E, Szakács G, Bezur L. Role of the EDTA Chelating Agent in Bioscouring of Cotton. Text Res J. 2005; 75(5):411-417.
43. Dongare KS, Shinde MR, Khyade VB. Mathematical Inverse Function (Equation) For Enzyme Kinetics. Res J Rec Sci. 2018; 7(12):1-7.
44. Lineweaver H and Burk D. The determination of enzyme dissociation constants. J Am Chem Soc. 1934; 56(3):658-666.
45. Sreenath HK, Koegel RG, Moldes AB, Jeffries TW, Straub RJ. Enzymic saccharification of alfalfa fibre after liquid hot water pretreatment. Proc Biochem. 1999; 35(1999):33-41.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

46. Lee CH. A Simple Outline of Methods for Protein Isolation and Purification. Endocrinol Metab. (Seoul). 2017; 32(1):18-22.

47. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959; 31 (3):426-428.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License