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Production and Optimization of Cellulase from *Penicillium* sp. Using Corn-cob and Pawpaw Fibre as Substrates

Francis Sopuruchukwu Ire[1*], Augustine Onwuchekwa Okoli[1,2] and Victor Ezebuiro[1,3]

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Nigeria.
[2]Department of Science Laboratory Technology, Federal Polytechnics, Oko, Anambra State, Nigeria.
[3]World Bank African Centre of Excellence, Centre for Oil Field Chemicals Research, Institute of Petroleum Studies, University of Port Harcourt, Choba, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author FSI designed the study, performed the statistical analysis while author AOO wrote the protocol. Author AOO managed the analyses of the study. Author VE managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study reports the production and optimization of cellulase from *Penicillium* sp. using corn-cob (CC) and pawpaw fibre (PF) as substrates.
Methods: Nine fungal isolates, obtained from compost soil, were screened for cellulolytic activity. Isolate CPF-1, based on its ability to give the highest zones of clearance and cellulolytic activity, was selected. CPF-1 was identified as *Penicillium* sp. based on its cultural and morphological characteristics. Cellulase activity was determined by the DNS method on Congo red agar plate. Effects of temperature, pH and metal ions (Zn^{2+}, Hg^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+} and Co^{2+}) on crude cellulase activity and stability were studied using two substrates (corn-cob and pawpaw fibre) by solid state fermentation.

**Corresponding author: E-mail: francis.ire@uniport.edu.ng, frankire4@yahoo.com;*

Results: Data obtained from the study revealed that the optimal pH and temperature values for the production of crude cellulase by the *Penicillium* sp. were pH 5 and 30°C, respectively; with maximum cellulase activity of 37.32 IU/mL. Optimum cellulase productivity of 15.787 IU/mL was obtained with CC as the substrate while 2.141 IU/mL was obtained with PF substrate after 1 h of fermentation. The cellulase produced was most stable at pH 5 and temperature of 40°C. Fe^{2+} and Co^{2+} were able to stimulate cellulase activity whereas the other ions inhibited the enzyme activity.
Conclusion: This study has revealed the potentials of corn-cob and pawpaw fibre as substrates for cellulase production by *Penicillium* sp. through solid state fermentation (SSF); with corn-cob as the most suitable substrate. Considering that these substrates are readily available, they present cheaper substrate alternatives for potential large-scale cellulase production.

Keywords: Cellulase; Penicillium sp.; corn-cob; pawpaw fibre; solid state fermentation.

1. INTRODUCTION

Recent interest by researchers to produce cellulase from cheaper substrate is justifiable. Industrially, cellulases are used in various economically relevant processes. They are widely applied in biotechnology industry and other industries that engage in food processing, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry. Cellulases belong to the glycoside hydrolase family and catalyse hydrolysis of glyosidic linkages, depolymerizing cellulose to fermentable sugars [1]. These fermentable sugars are used in many industrial processes such as biofuel and other chemical productions [2,3].

Fungi with cellulolytic abilities are widespread in the soil. They comprise soft rot fungi: *Aspergillus niger, A. nidulans, A. oryzae, A. terreus, Fusarium solani, F. oxysporum, Humicola insolens, H. grisea, Melanocarpus albomyces, Penicillium brasilianum, P. occ1itanis, P. decumbans, P. fumigosum, Trichoderma reesei, T. longibrachiatum, T. harzianum, Chaetomium cellulyticum, C. thermophilum, Neurospora crassa, Thermoascus aurantiacus, Mucor circinelloides, P. janthinellum, Paecilomyces inflatus, P. echinulatum, Trichoderma atroviride*; brown rot fungi: *Coniophora puteana, Lanzitestrabeum, Poria placenta, Tyromyces palustris, Fomitopsis* sp.; and white rot fungi: *Phanerochaete chrysosporium, Sporotrichum thermophile, Trametes versicolor, Agaricus arvensis, Pleurotus ostreatus, Phlebia gigantean* [4].

Cellulases obtained from aerobic fungi are particularly preferred in the industry. This is because they are extracellular, adaptive in nature, and usually secreted in large quantities during exponential phase. These aforementioned features distinguish aerobic fungal cellulases from bacterial as well as from anaerobic fungal cellulases. Anaerobic fungal cellulases exist as tight multi-enzyme complexes, often membrane-bound as cellulosomes, from which it is difficult to recover individual active enzyme species; hence, economically less important [5,6]. There are widespread reports of cellulolytic enzyme production from aerobic fungi. Some of the aerobic fungi reported for efficient cellulase production include members of the following genera: *Aspergillus* [7], *Penicillium* [8], and *Trichoderma* [9]. Fungal cellulases are structurally simple, with functionally distinct modules or domains [10].

Choice of substrate is an important consideration in cellulase production. Different substrates have been employed in the production of cellulase, and they range from pure cellulose [11] to diary manure [12]. Recently, agro residues have been used as carbon sources in cellulase fermentations. To make cellulase production economical, it has to be produced from readily available substrates and lignocellulolytic substrates offer this advantage. Many lignocellulosics have been reported for the production of cellulases. Amongst them include wheat bran, rice bran, corn cob [13], corn straw, corn stalk and husks, sugarcane bagasse and cassava peels [14,15,3], saw dust [8] etc.

In addition to substrates, the fermentation technology applied can greatly determine the success of cellulase production. Different fermentation technologies exist for the production of cellulases; however, the popularly used technologies are submerged and solid-state fermentations. Solid state fermentation (SSF) by fungi is a preferable production route for cellulase as it offers lower cost and enables the production of cellulase with higher titre [16]. Other advantages of SSF include maximum

productivity, ease of technique, low capital investment, low energy requirement and less water output, better product recovery, and lack of foam build-up. Moreover, SSF has been reported to be most appropriate cellulase production process for developing countries [17,18].

Furthermore, improved cellulase production requires the optimization of the fermentation conditions. Microbial cellulases are inducible in nature and their production can be influenced by several factors such as pH, temperature, agitation, substrate concentration, type of nitrogen and carbon source etc. Various metal ions and chemical compounds may also influence cellulase activity. However, metal ions such as Hg^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Ag^{+}, Mn^{2+}, K^{+}, may be slightly or completely inhibitory to cellulase, whereas other metal ions such as Ca^{2+}, Na^{+} and Co^{2+} stimulate the activity of cellulase [19,20,6]. This study investigated the production and optimization of cellulase from *Penicillium* sp. using corn-cob and pawpaw fibre as substrates.

2. MATERIALS AND METHODS

2.1 Pawpaw Fibre (PF) and Corn-cob (CC) Collection, Processing, Comminution and Pretreatment

The PF and CC used in this study were collected from the Department of Chemistry, University of Port Harcourt, Nigeria and processed using the method described by Ezebuiro et al. [15] with slight modification. In brief, the biomasses were washed and oven-dried at 130°C for 5 h. The dry biomasses were further grinded with an electric blender (Philips blender HR2001, Japan), filtered with a 60 Mesh (0.250 mm) sieve, stored in screw-cap glass containers and labelled accordingly for subsequent use.

Pretreatment was achieved by soaking method. Ten milligram (10 g) of each of the processed substrate was suspended in 200 mL of distilled water in a beaker and allowed to soak overnight. After soaking, each substrate was boiled for 5 min in 250 mL beaker to break the bonds of cellulose subunits, resulting in the release of simple sugars.

2.2 Isolation and Screening of Fungi for Cellulolytic Activity

The soil samples that served as source of fungus used in this study were collected from the bottom of decaying agricultural waste dumps within University of Port Harcourt, Choba, Nigeria. These soil samples were mixed together and crushed into small particles in a mortar. One gram of the soil mixture was suspended in 9 mL of physiological saline in a 250 mL beaker and stirred properly. The suspension was filtered with Whatman™ No 1 filter paper (Whatman, UK) and the resulting filtrate used for the isolation of cellulolytic fungi and yeasts.

The modified mineral medium of Nwodo-Chinedu [21] was used for the screening the fungal isolates. The medium contained: KH_2PO_4, 1.3 g; NH_4Cl, 0.5 g; and $MgSO_4.7H_2O$, 0.2 g in 1000 mL of distilled water. However, the medium was modified by adding $NaNO_3$, 1.5 g; and yeast extract, 0.5 g to supply nitrate and mineral elements, respectively. For each of the pretreated substrates, 2 g was taken and suspended in 25 mL of the mineral medium in 250 mL beaker (pH 5.0). The content was transferred to 100 mL conical flask with 5 mL of the soil filtrate added and mixed properly. A control flask (containing mineral medium without inoculum) was also set up. All culture flasks and the control were incubated at room temperature until growth of fungi appeared.

Serial dilution was performed according to the method described by Nanhini and Josephine [22]. Nine millilitres (9 mL) of normal saline (0.85 % NaCl in distilled water) was first dispensed into each clean test tube; the test tubes and their contents were sterilized in an autoclave at 121°C (15 psi) for 15 min. To prepare stock solution, 10 g of the dry soil sample was dissolved in 90 mL of sterile normal saline; from this stock solution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were made. Aliquots (0.1 mL each) from 10^{-3} to 10^{-6} dilutions were plated out onto sterile mineral salt agar, with 2 g of each substrate as sole source of carbon (the media were supplemented with ampicillin [100 µg/mL] to suppress the growth of bacteria). The inoculated plates were incubated at room temperature for 6 days until growth of fungi developed. Distinct colonies on the agar plates were transferred to freshly prepared PDA slants and stored at 4°C for further studies.

All nine isolates recovered from the soil samples were screened for cellulase production by spot-inoculating each of them on agar plates containing either CC or PF as sole carbon source. The plates were incubated for 5 days at room temperature. Thereafter, the inoculated plates were flooded with 0.1% (w/v) Congo red

for 30 min. The plates were destained with 1M NaCl. Cellulase-producing isolates produced clear zones around the cultures, indicating cellulase production [3]. The diameter of the halo zones produced was measured with a metre rule and recorded. The best isolate with the highest zone of clearance was chosen for further studies.

2.3 Determination of Enzyme Activity

The method of Miller [23] was employed in determining the enzyme activity assay. One millilitre of crude enzyme filtrate was added to 1 mL of 0.1% (w/v) carboxymethyl cellulose in a test tube. Sodium acetate buffer (0.1 mL, pH 6.5) was pipetted into the mixture and then incubated in a Water Bath (HH – W420, China) at 35 ^{o}C for 30 min. After incubation, 3 mL of the DNS reagent was added to terminate the reaction. The tube was boiled for 5 min and monitored for colour development. Optical density readings were taken at 546 nm and the sugar released extrapolated from the standard glucose curve. The result obtained was then expressed as cellulase activity (U/mL) defined as the enzyme that produced 1 µM of glucose per min under the assay conditions.

2.4 Protein Determination

Protein determination method used was a modification of Lowry et al. [24] method. In this method, colour formation was observed; colour formation arises from the reaction of alkaline copper with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. Precisely 0.2 mL of bovine serum albumin (BSA) working standard was pipetted into 5 mL test tubes and made up to 1 mL using distilled water. About 4.5 mL of Reagent I (48 mL of 2% Na_2CO_3 in 0.1 N NaOH, 1 mL of 1% NaK Tartrate in H_2O, and 1 mL 0.5% $CuSO_4.5H_2O$ in H_2O) was added and incubated for 10 min. After incubation, 0.5 mL of Reagent II (1 part Folin-Phenol [2 N]:1 part water) was added and incubated for 30 min. The absorbance was measured at 660 nm and the standard graph plotted. The amount of protein present in the given sample was estimated from the standard graph. Another test tube with 1 mL distilled water served as blank.

2.5 Effect of Temperature on Cellulase Production

Effect of different incubation temperatures (30, 40, 50, 60, 70 and 80°C) on the production of cellulase by the isolate was studied. This was done by incubating the culture medium at different temperatures and assaying the culture broth for cellulase activity.

2.6 Effect of Initial pH on Cellulase Production

Effect of different pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 8, and 9) on the production of cellulase by the isolate was studied by adjusting the pH of the culture medium and assaying the culture broth for cellulase activity.

2.7 Effect of Metal Ions on Cellulase Production

Effects of different cations, in their salt forms ($CaCl_2$, $MgSO_4$, $ZnSO_4.7H_2O$, Co $(NO)_2.6H_2O$, $HgCl_2$ and EDTA crystalline salt), on the production of cellulase by the isolate using PF and CC as substrates were examined. Each solution of the compound at 10 mM concentration was prepared with distilled water. The reaction mixture contained 1 mL of 1% (w/v) CMC in 1 mL of 0.05 M citrate buffer of pH 6.5 and 1 mL of salt of the respective cations or EDTA. Reaction mixtures were equilibrated to room temperature for 45 min and then inoculated with 0.5 mL of appropriate crude enzyme (from *Penicillium* sp.). The inoculated enzyme-substrate mixture was incubated at 50°C in a Water Bath for 30 min and 1 h, respectively. After incubation, the enzyme-substrate mixture was cooled for 12 min and cellulase activity determined by DNS method [23].

2.8 Effect of pH and Temperature on Cellulase Stability

Effect of pH on enzyme stability was performed as described by Nizamudeen and Bajaj [25] and Ponnuswamy and Prakash [26]. For pH stability study, 0.5 mL of the crude enzyme was pre-incubated without substrate; then, the buffer was adjusted to appropriate pH values at 50°C for 30 min and 1 h, respectively after which cellulase activity was determined.

To determine thermal stability of the enzyme, 0.5 mL of the crude cellulase was incubated without substrate at increasing temperatures of 30, 35, 40, 50, 60, 70 and 80°C for 30 min and 1 h, respectively and then assayed for cellulase activity.

2.9 Identification of the Cellulase-Producing Fungus

Benson et al. [27] fungal slide culture technique and the Colour Atlas for clinical fungi identification were used to identify the cellulase-producing fungus. The isolate was inoculated on the edge of sterilized 5 cm^2 block of PDA medium, cut out with sterile scalpel and placed on alcohol-sterilized glass slide. The inoculated agar block was covered with cover-slip and placed on top of the short piece of glass rod (8.9 cm) inside a petri dish whose bottom was lined with Whatman™ No 1 Qualitative filter paper. The filter paper was moistened with 5 mL of sterile distilled water to provide humid environment to the culture. The plate was covered and incubated at 30°C for 4 days. After incubation, the agar block culture was removed; the slide and cover-slip were stained with lactophenol cotton blue solution and examined under x40 and x10 objective lenses. Characteristics such as the presence or absence of conidia, phialides, septation and colour of mycelia were noted and used to identify the isolate.

3. RESULTS

3.1 Pawpaw Fibre (PF) and Corn-cob (CC) Collection, Processing, Comminution and Pretreatment

The PF and CC after processing and pretreatment generated usable substrates of size, 0.250 mm.

3.2 Isolation and Screening of Fungi for Cellulolytic Activity

Out of the nine fungal isolates screened for cellulolytic activity, CPF-1 was selected based on its ability to produce halo zones around the colonies when flooded with Congo red dye for 30 min and destained with 1M NaCl. The isolate also showed the highest activity when assayed for cellulase production.

3.3 Effects of Temperature, pH and Metal Ions on the Production of Cellulase and Release of Reducing Sugar

Effects of temperature, pH, and metal ions on the production of cellulase by *Penicillium* sp. utilising either CC or PF as substrates are presented in Fig. 1. *Penicillium* sp. released highest concentration of sugar (19.871 µg/mL) from corn-cob at pH 5.0 during hydrolysis of the substrate. The temperature optimum for cellulase production by *Penicillium* sp. using corn-cob as sole source of carbon was 30°C. At this temperature, *Penicillium* sp. yielded the highest glucose concentration of 3.2435 µg/mL. At higher temperatures (40 to 70°C), the release of glucose depreciated slightly for both substrates.

3.4 Effects of pH and Temperature on Crude Enzyme Stability

Effects of pH and temperature on the stability of the cellulase secreted by *Penicillium* sp. are presented in Fig. 2. From the figure, the cellulase was stable over a pH range of 4.5 to 6; being most stable at pH 5 with corn-con as the substrate. For temperature, the cellulase was stable over a temperature range of 30 to 80°C, with the highest stability observed at 40°C when corn-cob was used as substrate.

3.5 Protein Determination

Table 1 presents the protein concentration of the crude enzyme obtained from *Penicillium* sp. when grown on CC and PF substrates, respectively. From the table, the isolate yielded more protein with CC substrate than with PF.

3.6 Isolation and Identification of Organisms

The cellulolytic fungus selected for this study was isolate CPF-1 and was identified based on cultural and morphological features and microscopically, under x40 objective lens. *Penicillium* sp. produced blue-green to ash colonies on PDA plates (Plate 1). Microscopically, it had septate hyphae, branched conidiophores and brush-like conidial head.

Table 1. Protein estimation of the crude enzyme secreted by *Penicillium* sp. with different substrates

Substrate	OD_{745nm}	Protein concentration (μgmL^{-1})
Blank	0.00	0.00
Pawpaw	0.068	3.573
Corn-cob	0.4025	22.104

4. DISCUSSION

This study was carried out to investigate the production and optimization of cellulase from

Penicillium sp. using corn-cob (CC) and pawpaw fibre (PF) as substrates. Corn-cob, also known as cob of corn, is the central core of an ear of maize (*Zea mays*). It refers to the part of the ear on which the kernels grow. The chemical composition of corn-cob has been previously reported by Harini and Kumaresan [28]. Corn-cob can also be described as an agricultural lignocellulosic residue with high cellulosic content [28]. Similarly paw paw fibre is a lignocellulsic derived from paw paw (*Carica papaya*). Proximate chemical analysis of paw paw has been reported by Awe et al. [29]. These substrates were chosen because of their high carbohydrate (especially cellulose) content and their availability.

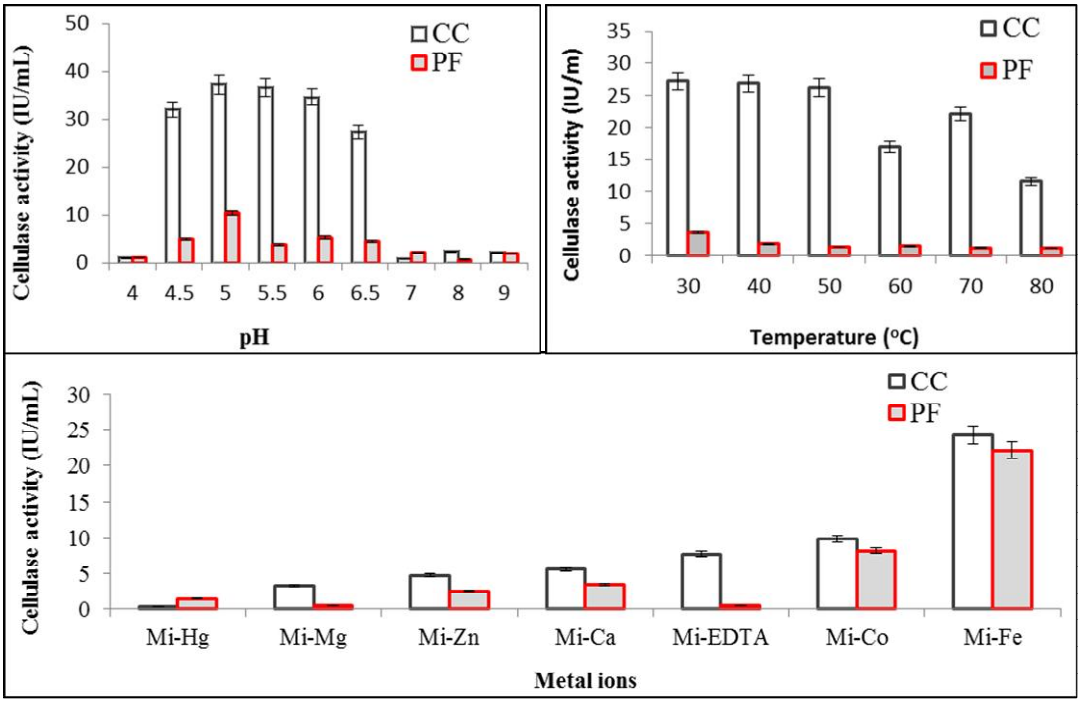

Fig. 1. Effects of pH, temperature, and metal ions on the activity of cellulase secreted by *Penicillium* sp. using different substrates

Legend: Mi-Hg = $HgCl_2$; Mi-Zn = $ZnSO_4$; Mi-Ca = $CaCl_2$; Mi-EDTA = EDTA; Mi-Mg = $MgSO_4$; Mi-Co = $Co(NO_3)_2.6H_20$; Mi-Fe = $FeSO_4.7H_2O$; CC = Corn-cob; PF = Pawpaw fibre

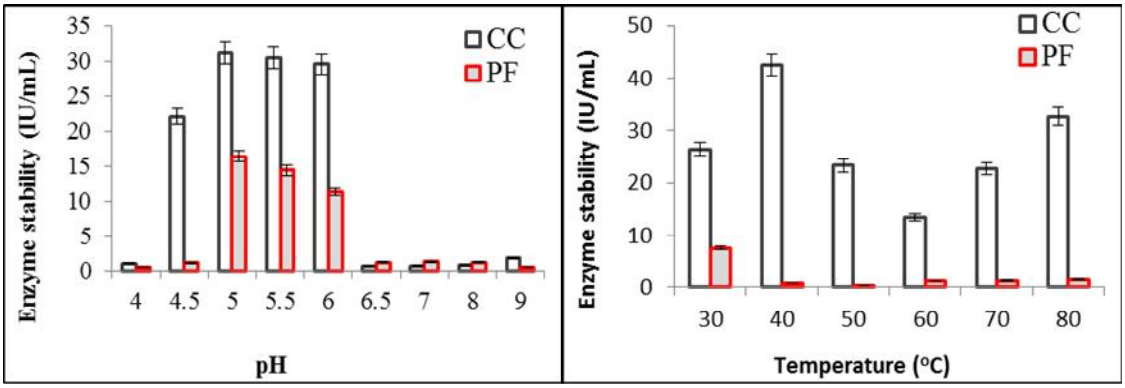

Fig. 2. Effects of pH and temperature on the stability of cellulase secreted by *Penicillium* sp. using different substrates

Legend: CC = Corn-cob; PF = Pawpaw fibre

Table 2. Optical density, sugar released, and cellulase activity from solid state fermentation of CC and PF substrates by *Penicillium* sp.

Parameters	CC substrate			PF substrate		
	OD_{546}	Sugar (µg mL^{-1})	CMCase (IU mL^{-1})	OD_{546}	Sugar (µg mL^{-1})	CMCase (IU mL^{-1})
pH						
4.0	0.025	0.602	1.034	0.037	0.911	1.565
4.5	0.613	15.759	27.085	0.035	0.859	1.476
5.0	0.773	19.871	34.150	0.007	0.124	0.213
5.5	0.758	19.497	33.508	0.023	0.537	0.923
6.0	0.725	18.647	32.048	0.034	0.833	1.432
6.5	0.319	8.180	14.059	0.019	0.434	0.745
7.0	0.021	0.486	0.835	0.022	0.524	0.901
8.0	0.035	0.859	1.476	0.017	0.395	0.679
9.0	0.048	1.194	2.052	0.034	0.821	1.410
Temperature						
30	0.1275	3.2435	5.574	0.048	1.181	2.030
40	0.044	1.0905	1.276	0.044	1.078	1.853
50	0.0205	0.4855	0.8345	0.061	1.529	2.628
60	0.032	0.782	1.344	0.022	0.524	0.901
70	0.031	0.756	1.2995	0.033	0.795	1.367
80	0.033	0.8075	1.388	0.040	0.988	1.698
Metal ions						
$HgCl_2$	0.011	1.240	0.412	0.036	0.885	1.521
$MgSO_4$	0.076	1.916	3.293	0.013	1.916	0.502
$ZnSO_4$	0.109	2.767	4.756	0.061	1.529	6.628
$CaCl_2$	0.128	3.256	5.596	0.08	2.019	3.47
EDTA	0.176	4.494	7.724	0.014	0.318	0.547
Co $(NO_3)_2.6H_2O$	0.224	5.731	9.850	0.187	4.777	8.210
$FeSO_4.7H_2O$	0.550	14.135	24.293	0.502	12.898	22.166

Values represent mean of fermentations at 30 min and 1 h, respectively.
Legend: OD_{546} – optical density at 546 nm

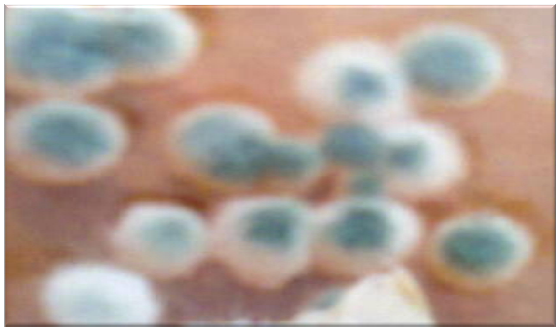

Plate 1. Cultural morphology of *Penicillium* sp. on PDA medium supplemented with corn-cob as sole carbon source

Colonies were greenish in colour with radiated ring on PDA plate; microscopic examination revealed branched conidiospores of long chains on conidia

Corn-cob (CC) used for cellulase production in this study is available locally and has been extensively reported [30-32] for use in cellulase production. However, there is dearth of information on the use of paw paw fibre (PF) in cellulase production. One of the uniqueness of this research is in its examination of locally available but scarcely researched substrate such as paw paw fibre for cellulase production. Although, corn-cob yielded more cellulase when compared to paw paw fibre as substrate, research on different pretreatment methods for paw paw fibre prior to solid state fermentation may yet improve cellulase production from paw paw fibre.

The effect of pH on cellulase production by *Penicillium* sp. was studied. The optimum pH for cellulase production was pH 5 while the fungus was able to secrete cellulase at pH range of 4.5 to 6.5. Similar optimum pH for cellulase production has been reported in other studies. Deshmukh et al. [33] reported pH 5 as the optimum pH for cellulase production by *Aspergillus niger subsp. awamori*. Similarly, Matsakas et al. [34] reported optimum pH of 5.5 by *Myceliophthora thermophilia* utilizing pure

cellulose. Ja'afaru and Fagade [35] reported maximum cellulase production at pH 5.0 by *A. niger* YL128; although they also reported significant levels of cellulase with other pH. Either low or high pH values may inactivates cellulase and thus affect its production. There is strong evidence that optimum pH for cellulase production varies amongst different organisms. pH 4.0 to 5.5 was reported for *A. terreus* by Garg and Neelakantan [36] and Pushelkar et al. [37]. pH 4.5 to 5.0 for *Rhizopus oryzae* and pH 5.0 to 6.0 for *Sclerotium rolfsii* have been reported by Darmwal [38] and Amadioha [39], respectively.

Temperature plays important role in the production of cellulase by microorganisms. The optimum temperature range for cellulase production in this study was between 30 and 50°C. Temperatures above 50°C were not suitable in enhancing cellulase production and activity. The result obtained in this study is similar to the findings of Ja'afaru and Fagade [34] who reported 30°C as optimum temperature for cellulase production by *A. niger* YL128. Their isolate was also able to secrete cellulase at a temperature range of 30 to 50°C. Ja'afaru and Fagade [34] reported that temperatures below 30°C and above 50°C may not be conducive for mycelial growth and cellulase synthesis. However, the result of this finding contradicts Matsakas et al. [33] report of highest cellulase activity at 65°C by *M. thermophilia*. More so, different cellulase-producing fungi thrive at different optimum temperatures.

The result obtained from the effect of various metal ions (Hg^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, EDTA, Co^{2+}, and Fe^{2+}) on the production of cellulase by *Penicillium* sp. showed that Fe^{2+} and Co^{2+} stimulated cellulase production; whereas, the other metal ions inhibited cellulase production. These findings are similar but not totally in agreement with the reports by Bagga et al. [40], Yan et al. [41], Dutta et al. [19], and Nazir et al. [42]. They reported that metal ions such as Hg^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Ag^{+}, Mn^{2+}, and K^{+} are slightly or completely inhibitory to cellulase production, whereas metal ions such as Ca^{2+}, Na^{+}, and Co^{2+} stimulate the activity of cellulase. The difference is that Ca^{2+} was not able to stimulate cellulase production in this present study. Meanwhile, Sajith et al. [6] reported differences in metal ions preference for cellulase production by different fungal isolates. Furthermore, Deshmukh et al. [33] reported that Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, and Fe^{2+}, all had positive influence on cellulolytic activity; although they used *Aspergillus niger* as the fermenting fungus.

5. CONCLUSION

This study revealed that corn-cob (CC) and pawpaw fibre (PF) present potential substrates for cellulase production by *Penicillium* sp. through solid state fermentation (SSF). This finding is particularly interesting considering that these substrates are readily available and present cheaper substrate alternatives for large-scale cellulase production. Moreover, the optimum cellulase productivity of 15.787 IU/mL and 2.141 IU/mL obtained with CC and PF as substrates indicate that these substrates may be suitable for large-scale cellulase production. In addition, the study revealed that pH, temperature, and metal ions influenced the ability of *Penicillium* sp. to secrete cellulase while utilising CC and PF as substrates. The cellulase was stable over broad range of temperature, which is an important characteristic for industrial scale production of cellulase. Thus, paw peels and corn cob present good substrates for the production of cellulase by *Penicillium* sp, especially at optimum cultural conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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