African Journal of Biochemistry Research Vol. 1 (1), pp. 006-010, June 2007
Available online at http://www.academicjournals.org/AJBR

Full Length Research Paper

Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by *Penicillium chrysogenum* PCL501

Nwodo-Chinedu, S.[1]*, Okochi, V. I.[2], Smith, H. A.[2], Okafor, U. A.[2], Onyegeme-Okerenta, B. M. and Omidiji, O.[3]

[1]Department of Biological Sciences, College of Science and Technology, Covenant University, KM 10 Idiroko Road, Canaan Land, PMB 1023 Ota, Ogun State, Nigeria.
[2]Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003 Idiaraba, Nigeria.
[3]Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos, Lagos State, Nigeria.

Accepted 15th May, 2007

The effects of glucose, crystalline cellulose and sawdust of *Mitragyna cilata* on the growth and cellulase production, inferred from cellulase (EC 3. 2. 1. 4) activity, of *Penicillium chrysogenum* PCL501 was determined. Glucose-containing media gave the highest mycelia weight of 1.78 mg mL^{-1} in 120 h of incubation. This is about 3.5 – 4.5 times the maximum weights of 0.51 and 0.40 mg mL^{-1} respectively obtained from the cultures containing cellulose and sawdust. The cultures containing crystalline cellulose and sawdust produced extracellular protein with cellulase (EC 3. 2. 1. 4) activity whereas glucose-containing cultures yielded very low protein and no significant cellulase activity. Maximum protein content of 0.02, 0.13 and 0.46 mg mL^{-1} respectively were obtained from the cultures containing glucose, cellulose and sawdust. Peak cellulase activity values of 100.0 and 92.2 Units L^{-1} respectively were obtained for the cultures containing cellulose and sawdust. There is a correlation between the protein released and cellulase activity of the culture filtrates. *P. chrysogenum* PCL501 produces extracellular proteins with significant cellulase activity in media containing cellulose and sawdust but not in glucose-containing medium. Sawdust is indicated as a good inducer of cellulase activity in the organism. The waste cellulosic material can be used as low-cost carbon source for commercial cellulase production.

Key words: *P. chrysogenum* PCL501, growth, cellulase activity, glucose, cellulosic materials.

INTRODUCTION

Plant biomass is a renewable resource available in tremendous quantities as agricultural, industrial and municipal wastes (Andren et al., 1975). Bioconversion of the lignocelluloses into animal feed-stock, bulk chemicals and biofuels is being studied as a means of alleviating food and energy shortages and reducing pollution-load (Howard et al., 2003; Saddler et al., 1982). Cellulase, a group of enzymes which catalyze the hydrolysis of cellulose and related cellooligosaccharide derivatives, is considered a potential tool for industrial saccharification of cellulosic biomass (Berry and Paterson, 1990), and an economic process for its production is thought to be critical for the successful utilization of cellulosic materials (Solomon et al., 1999; Wu and Lee, 1997). Complete enzymatic hydrolysis of native cellulose by *Penicillium pinophilum* was found to depend largely on the synergistic action of three types of the enzyme, namely, endoglucanase (EC 3. 2. 1. 4), exoglucanase (EC 3. 2. 1. 91) and β-glucosidase (EC 3. 2. 1. 21), (Wood and McCrae, 1986). Cellulase action is generally initiated by the random acting endoglucanases (Cellulase: EC 3. 2. 1. 4) at the amorphous regions within cellulose chain to produce cellooligosaccharides. The enzyme is adaptive in most fungi (Rapp et al., 1981; Reese and Levinson, 1952); substances such as cellulose and sophorose are known to stimulate its production (Mandels and Reese,

*Corresponding author. E-mail: sncresearch@yahoo.com. Tel: +234-802-8626605

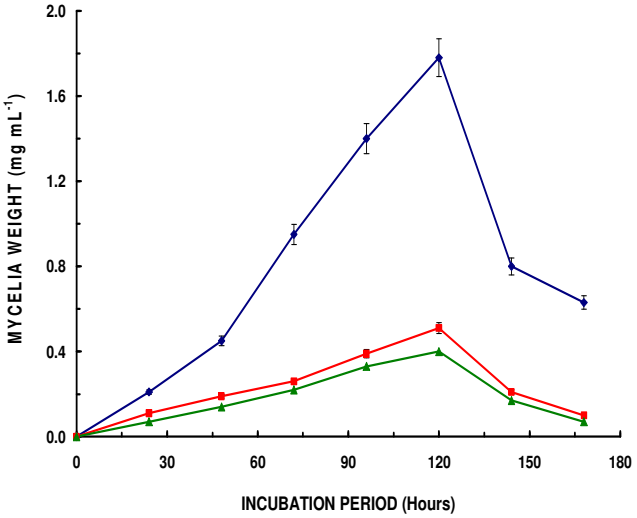

Figure 1. Mycelia weights of *Penicillium chrysogenum* PCL501cultured for 24 – 168 h at 30°C in media containing Glucose (♦), Cellulose (■) and Sawdust (▲) as sole carbon source.

1956; Ryu and Mandels, 1980). Many filamentous fungi secrete plant cell wall hydrolyzing enzymes such as cellulases and xylanases into their culture media which could be employed for the hydrolysis of lignocellulosic materials (Berry and Paterson, 1990). For example, *Penicillium chrysogenum* mycelium from penicillin manufacture is used as an additive in animal feeds to increase their content of proteins, sugars and mineral salts (Nuero and Reyes, 2002). Major impediments to the commercial use of cellulases are low activity and high production cost of the available enzyme preparations (Spano et al., 1978). This has necessitated the search for cellulolytic organisms with novel cellulase properties and strategies for low-cost enzyme production.

In search of viable cellulolytic organisms, we isolated from decomposing wood-wastes in Lagos, Nigeria, different cellulolytic microfungi which include a wild strain of *P. chrysogenum* PCL501; the culture supernatant of the filamentous fungus gave high cellulase activity (Nwodo-Chinedu et al., 2005). The organism also grows rapidly on media containing sugarcane pulp and sawdust as sole carbon sources with the result that basal media containing 2% of any of the two waste cellulosic materials was found adequate for its cultivation (Nwodo-Chinedu et al., 2007). In the present study, growth and cellulase production by the wild strain of *P. chrysogenum* PCL501 cultured in liquid media containing glucose, cellulose and sawdust were investigated. Our data shows high growth but insignificant cellulase production in glucose - containing media. This is in contrast to the cultures from media containing cellulose and sawdust which gave lower growth but significant cellulase production. There appears to be a correlation between protein content and cellulase activity of the cell-free filtrates.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. Potato Dextrose agar and crystalline cellulose were obtained from Merck, Germany. Carboxymethyl-Cellulose (CM52) was obtained from Whatman Limited, England. All other chemicals and reagents were obtained from Sigma Chemicals Co. Limited, England.

Cellulosic materials

Sawdust of Abora wood (*Mitragyna ciliata*) was collected from Okobaba Saw-mills, Ebute-Metta, Lagos, Nigeria. The sample was dried in the oven at 80°C for 2 h, ground with Marlex Exceller Grinder (Mumbai, India), and passed through a sieve of about 0.5 mm pore size to obtain the fine powder used for this study.

Media preparations

The liquid media (modified Czapek-Dox media) contained (g/ L): 3.0 $NaNO_3$, 0.5 KCl, 1.0 KH_2PO_4, 0.5 $MnSO_4$. $7H_2O_4$, 0.1 $FeSO_4.7H_2O$ and 1.0% carbon source (Glucose, Crystalline cellulose or sawdust). One liter (1 L) of the media was supplemented with 1.0 mL of trace metal solution containing (g/ L) 1.0 $ZnSO_4$ and 0.5 $CuSO_4.5H_2O$. The pH of each media was adjusted to 5.6 with 0.1M HCl before sterilizing in an autoclave at 121°C for 15 min.

Organism

The strain of *P. chrysogenum* PCL 501 used for this study was isolated and characterized as described previously (Nwodo-Chinedu et al., 2005). The organism was maintained at 4°C on Potato Dextrose Agar (PDA) slants. Fresh cultures were obtained by sub-culturing on fresh sterile PDA plates and incubating at 30°C for 72 h.

Growth studies

Fresh PDA cultures of the organism were prepared and incubated at 30°C for 72 – 120 h to obtain enough spores. Spores were collected by a modification of the method described by Vitale et al. (2002). Each plate was covered with 10 mL of 1.0% Tween 80 and spores were harvested into a sterile test tube using sterile cotton swab. Serial dilutions of the spore suspension were prepared in 1.0% Tween 80 and counted with a haematocytometer (Neubauer Counting Chamber, USA) mounted on light microscope. Two mL of the spore suspension (2.0 X 10^6 spores mL^{-1}) was inoculated into 100 mL of the respective sterile liquid media in 250 mL Erlenmeyer flask. The cultures were incubated at 30°C with continuous agitation using Griffin flask shaker and harvested by filtration using Whatman No. 1 filter paper at 24 h intervals for a period of 168 h. The mycelium was washed and dried in an oven at 80°C for 2 h. The cell-free filtrates were used for protein and enzyme analyses.

Protein assay

Protein content of the culture filtrates was determined by Lowry (1951) method using Bovine Serum Albumin (BSA) as standard.

Cellulase (EC 3. 2. 1. 4) assay

A modification of the reducing sugar method described by Khan (1980) was used to for the assay of endo-1, 4-β-Glucanase (EC 3.

2. 1. 4) activity. Carboxylmethyl-cellulose (CMC) was the enzyme substrate. The reaction mixture contained 2.0 mL of 0.1% (w/v) substrate in 0.1M sodium acetate buffer (pH 5.0) and 2.0 mL of cell-free culture supernatant. The mixture was incubated at 37°C in water bath for 30 min. The released reducing sugar was measured using 3, 5-dinitrosalicylic acid (Miller, 1959). The colour was developed by boiling in water bath for 5 min. Absorbance was read at 540 nm with Spectronic Genesys TMS (USA). Released reducing sugar was expressed in glucose equivalent. A unit of activity was defined as the amount of enzyme required to liberate 1µmol of glucose per minute under the assay conditions.

RESULTS

The growth of *P. chrysogenum* PCL501 in the liquid media containing glucose, cellulose and sawdust is shown in Figure 1. Peak mycelial growth in the three media was obtained at 120 hours. The medium containing glucose gave a very rapid growth with a peak mycelial weight of 1.78 mg mL^{-1}. The peak mycelial weights of 0.51 and 0.40 mg mL^{-1} respectively were obtained in the media containing cellulose and sawdust. Sharp decline in the mycelial weight was noted in all the media after the peak growth. Plots of the protein contents and cellulase activities of culture filtrates of *P. chrysogenum* PCL501 cultivated in the liquid media containing cellulose, sawdust and glucose respectively are shown in Figure 2. In cellulose-containing medium, both the protein content and cellulase activity increased steadily and peaked at the same period, and declined afterwards (Figure 2A). The peak protein content of 1.3 mg mL^{-1} and highest cellulase activity of 100.0 Units mL^{-1} (X $10^{3)}$ were obtained at 120 h from the cultures containing cellulose (Figure 2A). In the medium containing sawdust, the protein peak came before the cellulase activity peak (Figure 2B). The peak protein content of 4.6 mg mL^{-1} was obtained at 96 h of incubation whereas the maximum cellulase activity of 92.2 Units mL^{-1} (X $10^{3)}$ was obtained at 120 h (Figure 2B). The protein released in media containing sawdust was very high compared to that obtained in cellulose-containing media. Glucose-containing culture gave a very low protein levels with a peak value of 0.02 mg mL^{-1} at 72 h of incubation (Figure 2C). The cellulase activity obtained from the glucose-containing cultures was also very low with a maximum value of 6.2 Units mL^{-1} (X $10^{3)}$. The plots show a direct relationship between the protein content and cellulase activity of the culture filtrates.

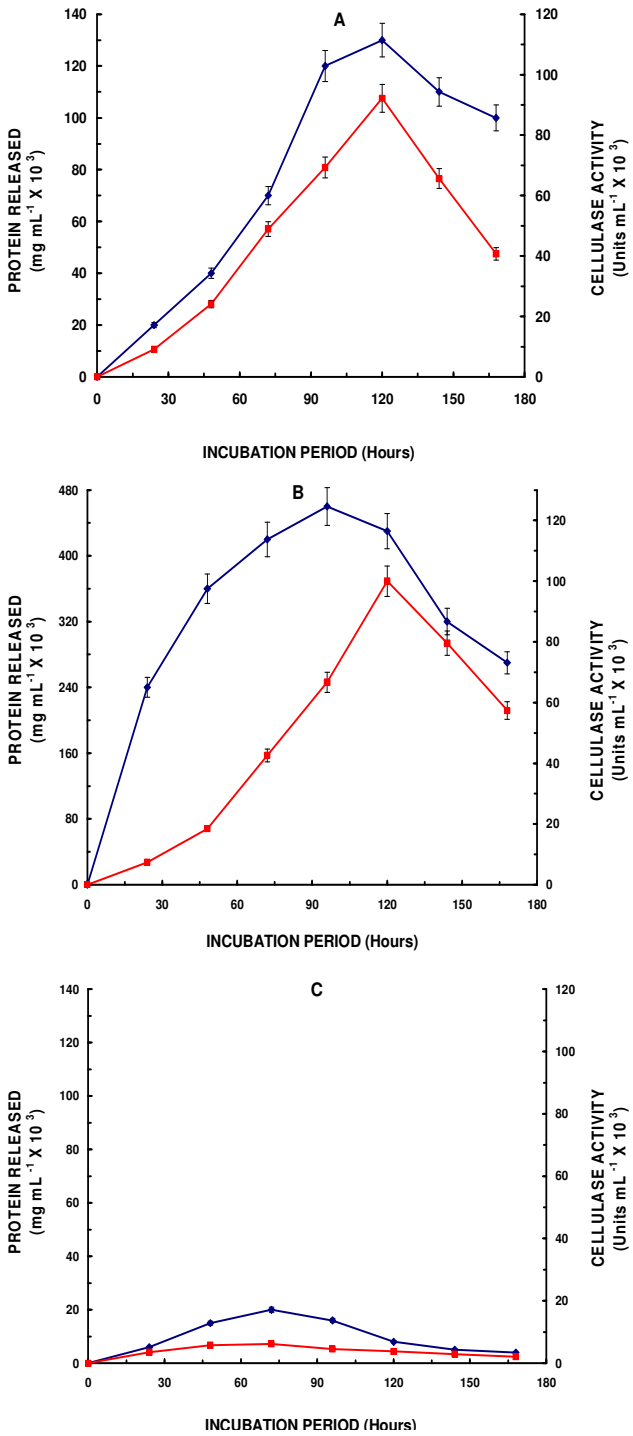

Figure 2. Protein contents (♦) and cellulase activities (■) of culture filtrates of *Penicillium chrysogenum* PCL501 incubated for 24 – 168 h at 30°C in liquid media containing (A) Cellulose, (B) Sawdust and (C) glucose.

DISCUSSIONS

The media containing glucose yielded higher amounts of mycelia compared to the media containing cellulose or sawdust as the respective carbon source (Figure 1). The differences in the complexity of the carbon sources could account for the disparity in the growth of the organism in the different media. In a study on the growth and β-galactosidase activity of the penicillin producer industrial *P. chrysogenum* NCAIM 00237 strain using different carbon sources, good growth was observed using glucose, sucrose, glycerol and galactose, while growth on lactose was

substantially slower (Nagy et al., 2001). Since glucose is more readily assimilated and metabolized by cells, there is greater tendency for organisms to grow very rapidly in media containing the simple sugar than that which contain cellulose or sawdust. Cellulose is a polymer of β-D-glucose while sawdust (wood) is composed of complex plant cell wall polymers which include cellulose, hemicelluloses and lignin (Grant and Long, 1981). In order to obtain simple sugars from cellulose or sawdust, the organism would have to synthesize cellulases and other enzymes required for the hydrolysis of the macromolecules. Cellobiose (a dimmer of β-D-glucose) and glucose are the major products of enzymatic hydrolysis of cellulose and sawdust (Unpublished). The cellulase activity and protein contents of filtrates from glucose containing media were extremely low and thus considered insignificant. This is because the organism already has the simple sugar, glucose, in its media and hence do not need to produce the hydrolytic enzymes (proteins). Cellulases and most plant cell-wall hydrolyzing enzymes are inducible and also regulated by catabolite repression in most fungi (Berry and Paterson, 1990). Presence of high concentration of glucose in the medium will thus turn off the production of the enzymes. This may account for the low protein content and insignificant cellulase activity recorded in culture filtrates from glucose-containing media. This goes on to show that the cellulase enzyme (EC 3. 2. 1. 4) of the wild-type *P. chrysogenum* PCL501 is inducible and that its production is stimulated by cellulose and sawdust in the absence of glucose. Rapp et al. (1981) reported that the endo-1, 4-β-glucanases of *Penicillium janthinellum* were found to be cell free, and their formation induced by cellobiose whereas the 1, 4-β-Glucosidases were primarily cell free, but with a small amount strongly associated with the cell wall, and were formed constitutively.

The organism has different periods for peak cellulase activity for the different carbon sources. The time was shorter when incubated on pure cellulose compared to sawdust. This may be as a result of the presence of other polymers such as the hemicelluloses in sawdust which can also furnish the organism with simple sugars. Most microorganisms generally have better ability to deploymerize hemicelluloses than cellulose due to the greater solubility of the former (Grant and Long, 1981). *P. chrysogenum* PCL501 was found to produce high levels of xylanases when cultured on media containing sawdust and other agro-wastes (Unpublished). The situation may be different with cellulose since it contains only β-D-glucose monomers. With cellulose as sole carbon source, production of cellulases may be more rapid because the glucose needed for the organism's metabolism must come from cellulose hydrolysis.

In terms of protein yield, higher values were obtained in media containing sawdust compared to that containing crystalline cellulose. The high protein released in the sawdust suggests the presence of other proteins (beside the cellulase enzyme) which may include many other cell-wall hydrolyzing enzymes. Secondary plant cell-walls, in addition to cellulose, contain other polymers, particularly hemicelluloses and lignin, which could induce the production of many degrading enzymes. Hemicellulases such as xylanase is required for the hydrolysis of natural cellulose (Khan, 1980).

There is an apparent correlation between the protein content and cellulase activity of the crude enzyme obtained at the different period of incubation. The organism seems to secrete the hydrolytic enzymes for the breakdown of the polymers into the growth media which largely accounts for the protein contents of the cell-free filtrates.

In conclusion, the wild strain of *P. chrysogenum* PCL501 produces extracellular proteins with significant cellulase activity in media containing cellulose and sawdust as sole carbon sources. On the other hand, the organism did not yield significant extracellular protein or cellulase activity with glucose as sole carbon source. Much more protein was produced on sawdust compared to cellulose. Sawdust is indicated as a good inducer of cellulase activity in *P. chrysogenum* PCL501. The waste cellulosic material is available in abundance and can be used as low-cost carbon source for the production of commercial cellulases. Such use could significantly reduce the production cost of cellulases as well as environmental pollution due to wood-wastes.

REFERENCES

Andren RK, Mandels MH, Mederios JB (1975). Production of sugars from waste cellulose by enzymatic hydrolysis 1: Primary evaluation of substrates. Proceedings of the 8th cellulose conference. Appl. Poly. Symp. 28: 205-219.

Berry DR, Paterson A (1990). Enzymes in Food Industry. In Enzyme Chemistry, Impact and applications, 2nd ed. CJ Suckling (Ed.). pp. 306-351.

Grant WD, Long PE (1981). The carbon cycle. In Environmental Microbiology, Tertiary level Biology, Thomas Litho Ltd., Scotland. pp. 91-116.

Howard RL, Abotsi E, Jansen Van Rensburg EL, Howard S (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. Review: Afri. J. Biotechnol. 2 (12): 602-619.

Khan AW (1980). Cellulolytic enzyme system of Activibrio cellulolyticus, a newly isolated Anaerobe J. Gen. Microbiol. 121: 499-502.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193: 265-275.

Mandels M, Reese ET (1956). Induction of cellulase in Trichoderma viride as influenced by carbon sources and metals. Biotechnol. Bioeneng. 73 (2): 269-278.

Miller GL (1959). Use of dinitrosalicyclic reagent for the determination of reducing sugars. Anal. Chem. 31: 426-428.

Nagy Z, Keresztessy Z, Szentirmai A, Biro S (2001). Carbon source regulation of beta-galactosidase biosynthesis in *Penicillium chrysogenum*. J. Basic Microbiol. 41 (6): 321-362.

Nwodo-Chinedu S, Okochi VI, Smith HA, Omidiji 0 (2005) Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. Int. J. Biomed Health Sci. 1(2): 41-51.

Nwodo-Chinedu S, Okochi VI, Omidiji O, Omowaye OO, Adeniji BR, Olukoju D, Chidozie F (2007). Potentials of cellulosic wastes in media formulation. Afri. J. Biotechnol. 6 (3): 243-246.

Nuero OM, Reyes F (2002). Enzymes for animal feeding from Penicil-

lium chrysogenum mycelial wastes from penicillin manufacture. Lett. Appl. Microbiol. 34: 413-416.
Reese ET, Levinson HS (1952). A comparative study of the breakdown of cellulose by microorganisms. Physiol. Plantarum 5: 345-366.
Ryu DD, Mandels M (1980). Cellulases: Biosynthesis and Applications. Enzyme Microbiol. Technol. 2: 92-102.
Solomon BO, Amigun B, Betiku E, Ojumu TV, Layokun SK (1999). Optimization of cellulase production by *Aspergillus flavus* Linn Isolate NSPR101 Grown on Bagasse. JNSCHE, 16: 61-68
Spano L, Alien A, Tarssinane T, Mandels M, Ryu DD (1978). Reassessment of economics of Technology for production of ethanol Proceedings in Industrial fuel from biomass symposium. Troy, New York. pp. 671-674
Vitale RG, Mouton JW, Afeltra J, Meis JFG, Verweij PE (2002). Method for measuring postantifungal effect in *Aspergillus* species. Antimicrob. Agents Chemother. 46 (6): 1960-1965.
Wood TM, McCrae SI (1986). The cellulase of *Penicillium pinophilum*: synergism between enzyme components in solubilizing cellulose with special reference to the involvement of two immunologically-distinct cellobiohydrolases. Biochem. J. 234: 93-99.
Wu Z, Lee YY (1997). Inhibition of the enzymatic hydrolysis of; cellulose by ethanol. Biotechnol. Lett. 19: 977-979.