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Original Article

Antibacterial Activity of Culture Extracts of *Penicillium chrysogenum* PCL501: Effects of Carbon Sources

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Abstract:

Penicillium chrysogenum PCL501 produced β-lactam antibiotics when fermented with different agro-wastes: cassava shavings, corncob, sawdust and sugarcane pulp. *In vitro* antibacterial activity of the culture extracts was tested against four clinical bacterial isolates, namely, *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. All the culture extracts and standard drug (commercial Benzyl Penicillin) inhibited the growth *B. subtilis* and *E. coli*; the potency varied with carbon source. Antibacterial activity of extracts from cultures containing cassava shavings and sugarcane pulp was comparable with that of the standard drug. The MIC against the susceptible organisms was 0.20mg/ml for the standard drug and ranged from 0.40 to 1.50mg/ml for the culture extracts. Neither the culture extracts nor the standard drug inhibited *K. pneumoniae* and *P. aeruginosa*; the bacterial strains produced β-lactamase enzymes. Cassava shavings and sugarcane pulp are indicated as suitable cheap carbon sources for the production of antibiotics by *Penicillium chrysogenum* PCL501.

Key Words: Agro-wastes, *Penicillium chrysogenum* PCL501, Antibiotic production, Phenyl penicillin, Antibacterial activity, β-lactamase

Introduction:

Penicillium chrysogenum (formerly, *Penicillium notatum*) is an important industrial organism due to its ability to produce several β-lactam antibiotics, particularly penicillins.[1] The chance discovery of *Penicillium notatum* by Alexander Fleming and the production of the revolutionary drug, penicillin, is perhaps the most important finding in the history of therapeutic medicine.[2] Two naturally occurring and commercially available penicillins are Benzyl penicillin (Penicillin G) and Phenoxy-methyl penicillin (Penicillin V). The R-group substituent of the penicillin nucleus can be substituted to give the molecule different antibacterial properties. The antibacterial effect of β-lactam antibiotics is effectively nullified by different types of bacteria which produce β-lactamase, an enzyme that breaks the β-lactam ring.[3] Clinical isolates of extended-spectrum β-lactamase (ESBL)-producing bacteria have been reported in different regions of the world.[4-6]

Efforts at improving penicillin yields have centred on growth optimization, development of available strains of *P. chrysogenum* by classical mutagenesis procedures, and the search for better strains of the organism.[7,8] Although these have led to the availability of cheaper and effective penicillins, further reduction in production cost of the antibiotics could be achieved by the use of low-cost fermentation substrates. Residual plant materials in urban refuse can serve as cheap carbon and energy sources for fermentation instead of refined sugars such as glucose and lactose.[9,10] This could turn the recalcitrant waste plant biomass into a valuable resource and reduce the pollution problem caused by its accumulation in the environment.[11,12] In Nigeria, agro-industrial wastes abound in the form of wood-wastes and crop residues such as cassava shavings, corncobs and sugarcane pulp.[13]

In this study, a strain of *P. chrysogenum* (PCL501) was fermented on four major waste cellulosic materials (cassava shavings, corncob, sawdust and sugarcane pulp) produced in Lagos, Nigeria and the culture extracts were tested for antibacterial activity against four clinical bacterial isolates (*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The fungus thrives on sawdust and sugarcane pulp[14] and produces hydrolytic enzymes such as cellulases[15] xylanases[16,17] and pectinases (unpublished data) in media containing agro-wastes. The present results show that the strain of *P. chrysogenum* (PCL501) produces effective β-lactam antibiotics with antibacterial activity comparable to commercial benzyl penicillin (Retarpen, Sandox, Austria). Two waste plant materials, cassava shavings and sugarcane pulp, are indicated as suitable low-cost substrates for the production of antibiotics by the strain of *P. chrysogenum*.

Materials and Methods:

Preparations of Agro-waste materials:

Sawdust of Abora wood (*Mitragyna ciliata*) was collected from sawmills at Ikorodu, Lagos, Nigeria. Mature sugarcane (*Saccharum offinarum*) stems, fresh maize (Zea mays) and cassava shavings (*Manihot esculenta*) were purchased from a local market in Mushin, Lagos, Nigeria. The crushed sugarcane pulp was soaked overnight and washed repeatedly in distilled water until no trace of simple sugar was detected. Corncob was obtained by removing the maize grains and cut into small sizes. The materials were separately dried at 80°C to constant weight in the oven, and milled using Marlex Exceller grinder (Mumbai, India). Fine powder obtained after passing each through a sieve of 0.5 mm pore size was used as substrate in the fermentation media.

Strain of *Penicillium chrysogenum* PCL501:

The strain of *P. chrysogenum* (PCL501) used for this study was isolated from a wood-waste dump in Lagos, Nigeria and characterized as described previously.[18] The organism was maintained on PDA slant at 4°C.

Growth culture and antibiotic production:

The fungus was sub-cultured on PDA plates and incubated at 30°C for 3-5 days to obtain the spores used for antibiotics production. Spores were washed into a sterile beaker using 0.1% Tween 80 in 0.1M potassium phosphate buffer at pH 7.0. The spore suspension was standardized such that 1 in 10 dilutions has an Absorbance of 0.48 at 530nm. Fifty millilitres (50 ml) of the spore suspension was aseptically introduced into a litre of sterile fermentation media containing per litre of distilled water: 6.0g Ammonium acetate, 0.5g $NaSO_3$, 0.02g $ZnSO_4.7H_2O$, 0.25g $MgSO_4.7H_2O$, 6.0g KH_2PO_4, 0.02g $FeSO_4.7H_2O$, 0.5g Phenylalanine, and 10.0g carbon source (glucose, lactose, cassava shavings, corncob, sawdust or sugarcane pulp). The pH was adjusted to 6.0. The flasks were incubated with intermittent shaking for 21 days after which the contents were sieved through cotton wool and filtrate centrifuged to remove cells. The pH of the supernatant was adjusted to 2.5 with chloroform-phosphate buffer (20:1) and assayed for Antibacterial activity.

Standard Reference Drug:

Benzyl penicillin (Retarpen, Sandox, Austria) was purchased from the University pharmacy at the college of Medicine, University of Lagos. Sixteen (16) mg/ml of the drug in 0.1M potassium phosphate buffer (pH 7) was used as standard reference drug for the *in vitro* tests.

Clinical Bacterial Isolates:

Clinical isolates of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* used for the study were provided by the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.

Antibacterial Sensitivity Testing:

Antibacterial activity of the culture extracts was assayed by a modified method of Grau and Halliday.[19] Nutrient agar plates were seeded with 0.1 ml of an overnight culture of each clinical isolate (equivalent to 10^7 – 10^8 CFU ml^{-1}). A sterile cork borer of 8 mm diameter was used to cut three uniform wells on the surface of the agar after 24 hours of incubation. The wells on each plate were then filled with 0.3 ml of a particular culture extract and the effect on the growing "lawns" of each clinical isolate was monitored at intervals of 24 hours. This was repeated for each culture extract and the standard drug. Zones of clearance round each well means inhibition and the diameter; such zones were measured after a marked decline in the potency of the antibiotics to inhibit the growth of the test organisms was noticed.

Determination of MIC

Minimum inhibitory concentration (MIC) was determined using the agar dilution guideline of NCCLS as described by Enwuru *et al.*[20] The concentrations with inhibitory zone diameter of 10.0 mm were chosen for the assay. Different dilutions of the extracts were prepared to give final concentration in the range of 2.0, 1.0, 0.8, 0.6, 0.4 and 0.2 mg ml^{-1} were prepared from a stock solution of 5mg ml^{-1}. One milliliter (1 ml) of each dilution was mixed with 18 ml of Mueller Hinton agar (MHA, Difco, France) and poured into Petri-dishes and allowed to set. The agar was streaked with an overnight broth culture of the clinical isolates (adjusted to turbidity equivalent to 0.5 McFarland standards) and incubated at 37°C for 24 hours. Controls containing only the nutrient agar and test organisms were set up. The plates were then examined for the presence or absence of growth. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each organism.

Tests for β-Lactamase activity:

The presence of β-lactamase enzyme in the clinical isolates was determined using β-lactamase identification sticks (Oxoid, Wesel, Germany) with nitrocefin as the substrate.[21] Cells from a 24-hour culture of each isolate were collected into a test tube and lysed by sonication. This was centrifuged at 4000g for 15 minutes. The supernatant diluted 1 in 100 with nutrient broth and a β-lactamase identification stick with nitrocefin as the substrate was inserted into it and allowed to stand for 30 minutes. Presence of β-lactamase was indicated by a colour change from light to deep pink.

Test for β-Lactam antibiotics

Culture extracts of *P. chrysogenum* PCL501 were tested to confirm if the antibacterial activity was due to the presence of β-lactam antibiotics. The supernatant containing β-Lactamase enzyme in the previous assay was used for the test. The supernatant (0.5 ml) was incubated with 0.5 ml of the culture extract and allowed to stand for 30 minutes at room temperature. The mixture was introduced into wells on agar plates streaked with β-Lactamase-free *E. coli* strain. The plates were observed for inhibition after 24 hours of incubation. Absence of noticeable zone of inhibition confirms the presence of β-lactam antibiotics.

Determination of Potency:

The potency of the extracts was determined by a modification of Carter's method as described below.[21] Three plates were used for each sample solution. On each plate were two cylinders were filled with the reference dose of standard of known concentration, two cylinders with the sample solution, and two cylinders with an aliquot of the sample solution that has been treated with the supernatant containing β-Lactamase enzyme. The plates were incubated overnight at 30°C and the diameters of the zones of inhibition were measured. A positive test for the presence of an antibiotic residue is indicated by the production of zones of inhibition by the sample solution and the absence of any detectable zones of inhibition by the β-lactamase-treated portion of the sample solution. The mean responses for the sample and the reference dose of standard were determined and the concentration of sample per milliliter of solution was calculated.

Results:

Plates 1–4 show the i*n vitro* effect of culture extracts of *Penicillium chrysogenum* PCL501 and Reference antibiotic (Penicillin G) on the clinical isolates of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The reference drug and all the culture extracts of *P. chrysogenum* inhibited the growth of *E. coli* and *Bacillus subtilis* but had no effect on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The summary of the antibacterial effect of the culture extracts and the reference drug on the clinical isolates is presented in Table 1. All the culture extracts and the reference drug exhibited antibacterial (positive) effects on *E. coli* and *Bacillus subtilis* and no antibacterial (negative) effect on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Table 2 shows the average zone of inhibition and potency of the culture extracts of *Penicillium chrysogenum* PCL501 and the reference drug against the clinical isolates of *Escherichia coli* and *Bacillus subtilis*. The degree of inhibition on the growth of the susceptible clinical isolates varied. The diameter of zone of inhibition and potency of culture extracts from sugarcane pulp and cassava shavings were very close to that of the reference drug. Table 3 shows the minimum inhibitory concentrations (MICs) of the culture extracts and the reference drug. The MIC against the susceptible organisms was 0.20mg/ml for the standard drug; it ranged from 0.40 to 1.50mg/ml for the culture extracts. MIC was 0.4 - 0.6mg/ml for cassava shavings and sugarcane pulp, 0.6mg/ml for glucose and lactose, 0.8mg/ml for corncob and 1.50mg/ml for sawdust

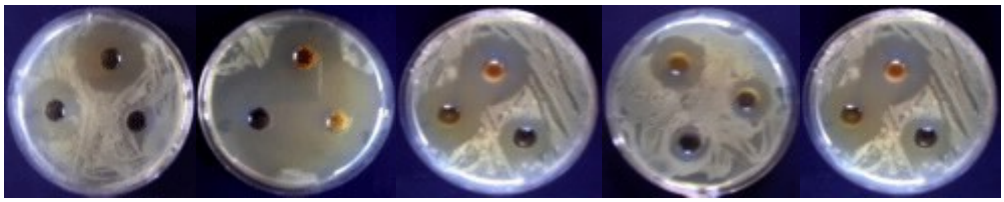

Plate 1 (A–E): *In vitro* effect of culture extracts of *Penicillium chrysogenum* PCL501 and the Reference antibiotic on the growth of clinical isolate of *Escherichia coli*. (Carbon Source: A = cassava shaving, B = sugarcane pulp, C = corncob, D = lactose; E = Reference drug)

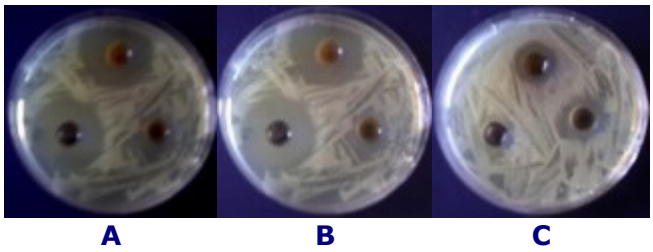

Plate 2 (A–C): In vitro effect of some culture extracts of *Penicillium chrysogenum* PCL501 on the growth of clinical isolate of Bacillus subtilis. (Carbon Source: A = cassava shaving; B = sugarcane pulp, C = sawdust).

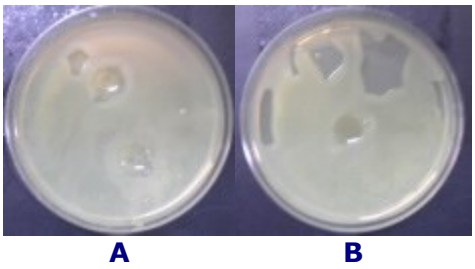

Plate 3 (A – B): In vitro effect of culture extracts of *Penicillium chrysogenum* PCL501 and the Reference antibiotic on the growth of the clinical isolate of Pseudomonas aeruginosa. (Carbon Source: A = corncob; B = Reference drug).

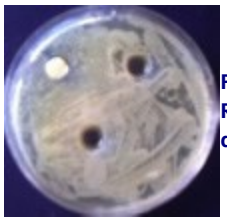

Plate 4: In vitro effect of some culture extracts of *Penicillium chrysogenum* PCL501 and the Reference antibiotic on the clinical isolate of Klebsiella pneumoniae. (Clockwise: Reference drug [white], cassava shavings and sugarcane pulp).

Table 1: Effect of culture extracts of *Penicillium chrysogenum* PCL501 and Reference drug on the growth of the clinical isolates of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (+ = inhibition; - = No visible zone of inhibition)

Antibiotic	Carbon Source of culture media	Antibacterial activity on clinical isolates of bacteria			
		Escherichia coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Culture extracts of *Penicillium chrysogenum* PCL501	Cassava shavings	+	+	-	-
	Corncob	+	+	-	-
	Sawdust	+	+	-	-
	Sugarcane pulp	+	+	-	-
	Glucose	+	+	-	-
	Lactose	+	+	-	-
Reference antibiotic (Penicillin G)	Not applicable	+	+	-	-

Table 2: Average diameter of inhibition zone and potency of culture extracts of *Penicillium chrysogenum* PCL501 and Reference drug against clinical isolates of *Escherichia coli* and *Bacillus subtilis*.

Source of Antibiotics	Carbon Source of culture media	*Escherichia coli*		*Bacillus subtilis*	
		Diameter of inhibition zone (mm)	Potency of antibiotic (mg/ml)	Diameter of inhibition zone (mm)	Potency of antibiotic (mg/ml)
Culture extract of *Penicillium chrysogenum* PCL501	Cassava shavings	15.12 ± 0.03	11.76	26.6 ± 0.14	10.43
	Corncob	13.63 ± 1.59	11.50	14.75 ± 0.35	5.47
	Sawdust	6.25 ± 0.35	3.04	6.75 ± 0.35	3.57
	Sugarcane pulp	17.88 ± 0.35	17.95	28.25 ± 1.77	11.41
	Glucose	15.00 ± 0.35	8.26	15.75 ± 1.06	5.78
	Lactose	14.75 ± 1.41	11.04	17.25 ± 1.06	6.27
Reference antibiotic (Penicillin G)	Not applicable	16.93 ± 0.50	16.00	34.46 ± 0.50	16.00

Table 3: Minimum inhibitory concentrations (MICs) of the culture extracts and the reference drug against the clinical isolates of *Escherichia coli* and *Bacillus subtilis*

Source of Antibiotics	Carbon Source of culture media	Minimum Inhibitory Concentration (mg/ ml)	
		Escherichia coli	*Bacillus subtilis*
Culture extract of *Penicillium chrysogenum* PCL501	Cassava shavings	0.60	0.40
	Corncob	0.80	0.80
	Sawdust	1.50	1.50
	Sugarcane pulp	0.60	0.40
	Glucose	0.60	0.60
	Lactose	0.60	0.60
Reference antibiotic (Penicillin G)	Not applicable	0.20	0.20

Discussion

The culture extracts of *P. chrysogenum* PCL501 exhibited significant antibacterial effects against clinical isolates of *E. coli* and *B. subtilis*. The results show that the organism produces antibiotics that are effective against the two bacterial isolates. This is consistent with the fact that most strains of *Penicillium chrysogenum* produce β-lactam antibiotics, mainly penicillins.[1] The fact that the antibacterial effect of some of the extracts is comparable with that obtained with commercial benzyl penicillin (Retarpen, Sandox, Austria) indicate the prospect of *P. chrysogenum* (PCL501) as potential source of commercial antibiotics. This is evident in the diameter of clearance zone, potency and MICs of the culture extracts.

It is interesting to note that the organism produced effective antibiotics in media containing refined sugars (glucose and lactose) as well as those containing the agro-wastes (cassava shavings, sugarcane pulp, corncob and sawdust) as sole carbon source. Most studies on suitable substrates for *Penicillium* fermentation had centered on the use of refined substrates such as glucose, sucrose, glycerol, galactose and lactose.[22] The present result indicates that the waste cellulosic materials are good substrates for antibiotic production by *P. chrysogenum* PCL501. Several studies have shown that the organism thrives on waste plant materials and utilizes the structural carbohydrates for its growth.[14,15,23] The organism is known to produce a variety of hydrolytic en-

zymes[24], particularly cellulases[15] and xylanases[17,25] which hydrolyze plant cell wall polysaccharides thereby enabling it to utilize the polymers for its carbon and energy needs.

Culture extracts from media containing two agro-wastes, cassava shavings and sugarcane pulp, yielded greater antibacterial effects than culture extracts from media containing equivalent amount of glucose and lactose. This indicates that the agro-wastes can effectively serve as cheap fermentation substrates for the production of antibiotics by the strain of *Penicillium chrysogenum*. Nigeria ranks among the highest producer of cassava tubers in the world and cassava shavings is a common domestic and agro-industrial waste in the country. Sugarcane is also produced and consumed in large quantity in the country. Sugarcane pulp (bagasse) comes from the consumption of sugarcane by the local community and as a byproduct of the sugar industries. The use of these materials, which are abundant in rural and urban refuse, in antibiotic production will not only lead to a reduction in the production cost of penicillin but also a reduction in pollution-load.

The Results show that both the standard penicillin G and culture extracts are not effective against the clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The two bacteria were found to produce β-lactamase enzymes which cleave the β-lactam ring and thereby nullify the effect of the β-lactam antibiotics. The beta-lactam ring is part of the structure of β-lactam antibiotics, principally the penicillins, cephalosporins, carbapenems and monolactams. This implies that *P. chrysogenum* PCL501 produces β-lactam antibiotics. Several strains of *Pseudomonas aeruginosa* have been reported to produce β-lactamases.[3,26,27] Strains of *E. coli* and *K. pneumoniae* producing β-lactamase have also been isolated in different hospitals.[5,6] This also confirms the presence of β-lactamase producing bacteria amongst clinical isolates from Nigerian. Since the resistance of the organisms to antibacterial drugs is both innate and plasmid-borne[28], there could be a high rate of the transference of such properties amongst bacterial strains. This calls for a review of the antibacterial regimes engaged in the treatment of bacterial infections. A combination of β-lactamase inhibitors with the β-lactam antibiotics is recommended to tackle the problem of the resistance caused by the presence of β-lactamases from bacterial cells.

References:

1. De Hoog GS, Guarro J, Figueras MJ, Gené J. *Atlas of Clinical Fungi. 2nd ed.* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and Universitat Rovira i Virgili, Reus, Spain, 2000; p. 1124.
2. Volk TJ. Tom Volk's Fungus of the Month for November 2003. TomVolkFungi.net, University of Wisconsin-La Crosse.
3. Naas T, Naas T, Phiippon L, Poirel L, Ronco E, Nordmann P. An SHV-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999;43:1281-4.
4. Baraniak A, Fiett J, Sulikowska A, Hryniewicz W, Gniadkowski M. Countrywide Spread of CTX-M-3 extended-spectrum ß-lactamase-producing microorganisms of the Family *Enterobacteriaceae* in Poland. *Antimicrol Agents and Chemother* 2002;46;151-159.
5. De Cueto M, López L, Hernández JR, Morillo C, Pascual A. In Vitro Activity of Fosfomycin against Extended-Spectrum-β-Lactamase- Producing *Escherichia coli* and *Klebsiella pneumoniae*: Comparison of Susceptibility Testing Procedures. *Antimicrob Agents Chemother.* 2006;50(1):368–370. doi: 10.1128/AAC.
6. Hernandez JR, Pascual A, Canton R, Martinez-Martinez L. Extended-spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Spanish hospitals (GEIH-BLEE project 2002). *Enferm. Infecc. Microbiol. Clin.* 2003;21:77-82.
7. Chuan-Bao., Sun Qiu-Lian, Kong Wen-Si Xu. Efficient transformation of *Penicillium chrysogenum* mediated by *Agrobacterium tumefaciens* LBA4404 for cloning of *Vitreoscilla* hemoglobin gene. EJB *Electronic J Biotechnol.* 2002;5(1):0717-3458.
8. Veenhuis M. Penicillin production by *Penicillium chrysogenum*: role of the microbody membrane in the penicillin biosynthetic pathway. *EJB Electronic Molecular Biology and Genetics J Biotechnol.* 2002;5;717-3458.
9. Howard RLI, Abotsi E, Jansen Van Rensburg, ELI, Howard S. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Review. *Afri. J. Biotechnol.* 2003;2(12):602-619.
10. Solomon BO, Amigun B, Betiku E, Ojumu TV, Layokun SK. Optimization of cellulase production by Aspergillus flavus Linn Isolate NSPR101 Grown on Bagasse. *JNSCHE*, 1999;16:61-68
11. Adney WS, Rivard CJ, Ming SA, Himmel ME. Anaerobic Digestion of Lignocellulosic Biomass and Wastes. Cellulases and Related Enzymes. *Appl. Biochem. Biotechnol.* 1991;30:165-183.
12. Rahman AMTF, Kyesmu PM, Tom TU. Biodegradation of wheat and corn offal for poultry feed production. Proceedings of the International Conference on Biotechnology: Commercialization and Food security. Abuja, Nigeria, 2000. p. 63-71.
13. Abu EA, Onyenekwe PC, Ameh DA, Agbaji AS, Ado SA. Cellulase (EC 3.2.1.3) production from sorghum bran by *Aspergillus niger* SL1: An as-

sessment of pretreatment methods. Proceedings of the International Conference on Biotechnology: Commercialization and Food security. Abuja, Nigeria, 2000. p. 153-157.
14. Nwodo-Chinedu S, Okochi VI, Omidiji O, Omowaye OO, Adeniji BR, Olukoju D, Chidozie F. Potentials of cellulosic wastes in media formulation. *Afr. J. Biotechnol.* 2007;6(3):243-246.
15. Nwodo-Chinedu S, Okochi VI, Smith HA, Okafor UA, Onyegeme-Okerenta BM, Omidiji O. Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by wild-type *Penicillium chrysogenum* PCL501. *Afri. J. Biochem. Res.* 2007;1(1):6-10.
16. Chinedu Nwodo S, Okafor UA, Emezue TN, Okochi VI. Xylanase production of *Aspergillus niger* and *Penicillium chrysogenum* from ammonia pretreated cellulosic waste. *Res. J. Microbiol.* 2008;3(4):246-253.
17. Okafor UA, Emezue TN, Okochi VI, Onyegeme-Okerenta BM, Nwodo-Chinedu S. Xylanase production by *Penicillium chrysogenum* PCL501 fermented on cellulosic wastes. *Afr. J. Biochem. Res.* 2007;1(4): 48-53.
18. Nwodo-Chinedu S, Okochi VI, Smith HA, Omidiji O. Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. *Int. J. Biomed Hlth Sci.* 2005;1(2):41-51.
19. Grau FH, Halliday WJ. The Effect of Nutrients on Formation of Penicillin by Washed Mycelium of Penicillium chrysogenum. Department of Bacteriology, University of Queensland, Brisbane, Australia. 1957;69:207.
20. Enwuru NV, Ogbonnia SO, Nkemehule F, Enwuru CA, Tolani O. Evaluation of antibacterial activity and acute toxicity of the hydroethanolic extract of *Stachytarpheta angustifolia* (Mill) Vahl**.** *Afri. J Biotechnol.* 2008;7(11):1740-1744
21. Carter GG. A review of procedures for the detection of residual penicillins in drugs. National Center for Antibiotics Analysis, Bureau of Drugs, FDA, BY-Lines 1977; 8.
22. Nagy Z, Keresztessy Z, Szentirmai A, Biro S. Carbon source regulation of beta galactosidase biosynthesis in *Penicillium chrysogenum*. *J. Basic Microbiol.* 2002;41(6):321-362.
23. Onyegeme-Okerenta BM, Nwodo Chinedu S, Okochi VI, Okafor UA. Agro-waste: potential substrates for *Penicillium chrysogenum*. *Int. J. Biol. Chem. Sci.* 2009;3(2):203-208.
24. Nuero OM, Reyes F. Enzymes for animal feeding from Penicillium chrysogenum mycelial wastes from penicillin manufacture. *Lett. Appl. Microbiol.* 2002;34:413-416.
25. Chinedu Nwodo S, Okafor UA, Emezue TN, Okochi VI. Xylanase production of *Aspergillus niger* and *Penicillium chrysogenum* from ammonia pretreated cellulosic waste. *Res. J. Microbiol.* 2008;3(4):246-253.
26. Iyobe S, Kusadokoro H, Takahashi A, Yomoda S, Okubo T, Nakamura A, O'Hara K. Detection of a Variant Metallo-ß-Lactamase, IMP-10, from Two Unrelated Strains of *Pseudomonas aeruginosa* and an Alcaligenes xylosoxidans Strain. *Antimicrobial Agents and Chemotherapy* 2002;46 (6);2014-2016.
27. Mugnier P, Dubrous P, Casin I, Arlet G, Collatz E. A TEM-derived extended-spectrum β lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1996;40;2488-93.
28. Shahid M, Malik A, Sheeba. Multidrug resistant *Pseudomonas aeruginosa* strains harbouring R-plasmids and Amp C β-lactamases isolated from hospitalized burn patients in tertiary care hospital of North India. *FEMS Lett* 2003;228:181-6.