African Journal of Biotechnology Vol. 9(7), pp. 1074-1078, 15 February, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1598
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Production of cellulolytic and xylanolytic enzymes by a phytopathogenic *Myrothecium roridum* and some avirulent fungal isolates from water hyacinth

Wahab Oluwanisola Okunowo[1]*, George Olabode Gbenle[1], Akinniyi Adediran Osuntoki[1], Adedotun Adeyinka Adekunle[2] and Sikiru Abiola Ojokuku[1]

[1]Department of Biochemistry, College of Medicine, University of Lagos, Lagos State, Nigeria.
[2]Botany and Microbiology Department, University of Lagos, Akoka, Lagos State, Nigeria.

Accepted 18 January, 2010

The cellulolytic and xylanolytic activity of a pathogenic *Myrothecium roridum* Tode (IMI 394934) and non-pathogenic *Fusarium solani* and *Curvularia pallescence* Boedjin isolates from water hyacinth were investigated. The mycelial plugs of each isolate was grown in submerged cultures of Czapeck Dox broth containing the appropriate carbon source (carboxymethylcellulose, sawdust and homogenized dry water hyacinth leaf) at 25°C for 16 days. The enzyme activity assay was carried out on the culture filtrates obtained. This was measured as micromole sugar released per min. The result obtained showed that the enzyme activity (U/ml) for β-1,4-exoglucanase, β-1,4-endoglucanase and xylanase was maximum 3.70 ± 0.43, 0.95 ± 0.03 and 2.32 ± 0.10, respectively, in *C. pallescens* Boedjin grown on carboxymethylcellulose and minimum 0.12 ± 0.02, 0.13 ± 0.03 and 0.34 ± 0.01 respectively, in *M. roridum* grown on homogenized dry water hyacinth leaf. The β-glucosidase activity (U/ml) was highest, 1.74 ± 0.06 in *M. roridum* grown on sawdust and least, 0.08 ± 0.00 in *C. pallescens* Boedjin grown on homogenized water hyacinth leaf broth. The maximum (324.00 ± 19.51 μg/ml) and minimum (130.00 ± 5.83 μg/ml) total extracellular protein was produced in *M. roridum* grown on homogenized dry water hyacinth leaf and carboxymethylcellulose, respectively. This study showed that the phytopathogenic strain of *M. roridum* is capable of producing cellulases and xylanase enzyme in submerged cultures but to a lesser degree compared to *F. solani* and *C. pallescence* Boedjin.

Key words: Cellulase enzymes, *Curvularia pallescence* Boedjin, *Fusarium solani, Myrothecium roridum*, Phytopathogens.

INTRODUCTION

Plant biomass is made up of mostly polysaccharides. The most abundant organic polysaccharide in the biosphere is cellulose (Murai et al., 1998; Hong et al., 2001; Narasimha et al., 2006) and is the major polysaccharide found in the plant cell wall giving the structural rigidity and strength to plants. Cellulose is an unbranched glucose polymer composed of β-1,4-glucose units linked by a β-1,4-D-glycosidic bond (Gielkens et al., 1999; Han et al., 1995).

A number of plant pathogenic organisms are capable of producing multiple groups of enzymes, called cellulases, that act to hydrolyze the β-1,4-D-glycosidic bonds within the cellulose molecules (Riou et al., 1991; Akiba et al., 1995; Baer and Gudmestad, 1995; Zaldivar et al., 2001; Moreira et al., 2005).

The cellulases are classified into three types: (i) endoglucanases or carboxymethyl cellulases (CMCases) [1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4)] (Lee et al., 2002), (ii) exoglucanases, including 1,4-β-D-glucan glucanohydrolases (also known as cellodextrinases) (EC 3.2.1.74) and 1,4-β-Dglucan cellobiohydrolases (cellobiohydrolases) (EC 3.2.1.91) and (iii) β- or β-glucoside glucohydrolases (EC 3.2.1.21) (Gielkens et al., 1999; Kang et al., 1999; Parry et al., 1983; Lee et al., 2002).

*Corresponding author. E-mail: modelprof@yahoo.com

Endoglucanases cut at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends (Siddiqui et al., 1999). Exoglucanases act by hydrolyzing the reducing or nonreducing ends of cellulose polysaccharide chains, liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products (Akiba et al., 1995; Han et al., 1995; Teeri, 1997; Lee et al., 2002). β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose (Lee et al., 2002).

The potential biotechnological applications of these enzymes in food and pharmaceutical industries, essential oils, pulp and paper industries, biomass conversion of agricultural and industrial wastes to chemical feedstock, biofuels, animal feeds and pollution control are well documented (Viikari et al., 1994; Christov et al., 1999; Zaldiva, 2001; Ikram-ul-Haq et al., 2006; Tarek and Nagwa, 2007; Acharya et al., 2008).

Cellulases and hemicellulases (such as xylanase) are produced by a wide range of microorganisms particularly fungi (Jorgensen et al., 2003).

A recent report showed the isolation of three fungal isolates (*Fusarium solani, Curvularia pallescence* Boedjin and *Myrothecium roridum* Tode) from water hyacinth. Of the three isolates, only the strain of *M. roridum* (IMI 394934) was pathogenic to water hyacinth and produced a phytotoxic metabolite which induced similar disease symptoms as the fungus on water hyacinth (Okunowo et al., 2008 a,b). The aim of the present study is to investigate the ability of these isolates to produce cellulolytic enzymes.

MATERIALS AND METHODS

Fungal isolate

The fungal isolates, *M. roridum* (IMI 394934), *C. pallescens* Boedijn and *F. solani* used in this study were obtained from water hyacinth in our previous study. The lyophilized sample of the organisms were reactivated and produced on potato dextrose agar (Okunowo and Ogunkanmi, 2009).

Media formulation and growth of isolates for enzyme production

Cellulolytic enzymes production in submerged cultures by the fungal strains was determined using three carbon sources; carboxymethylcellulose (substitution degree 0.7, Sigma), sawdust of Abora wood (*Mitragyna ciliata*) collected from sawmills at Ikorodu, Lagos, Nigeria and water hyacinth leaf (*Eichhornia crassipes*) collected from the University of Lagos Lagoon. The sawdust and water hyacinth leaf were washed in distilled water, dried at 70°C in an oven (SD 93114624, Gallenkamp, United Kingdom) and then pulverized using Marlex Exceller grinder (Mumbai, India). The pulverized samples were sieved through a mesh of 0.05 mm pore size to obtain a fine powder. Czapeck Dox broth (sodium nitrate 2 g, potassium nitrate 1 g, potassium chloride 0.5 g, magnesium sulphate 0.5 g, ferrous sulphate 0.01 g, sucrose 30 g) was formulated such that its sucrose was substituted with equivalent amount (30 g/L of distilled water) of the appropriate carbon source. Four mycelial plugs of 10 mm diameter cork borer were grown on the formulated Czapeck Dox broth and incubated at 25°C for 16 days. Aliquots were centrifuged at 12,000 x g to obtain supernatant for enzyme assay. The enzyme activity (Unit) was measured as micromole sugar released per min.

β-1,4-Endoglucanase activity

The β-1,4-endoglucanase activity was determined according to Zaldivar et al. (2001), using carboxymethylcellulose as substrate and the formation of reducing sugars was measured by reaction with dinitrosalicylic acid (DNS). The reaction mixtures containing 10 mg CMC (Carboxymethyl cellulose) in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml culture supernatant were incubated at 50°C for 30 min. The reducing sugar formed was measured with dinitrosalicyclic acid (DNS). One milliliter (1 ml) of DNS reagent was added to 3 ml of the test sample. The colour was developed by boiling the mixture in water bath for 5 min. Absorbance was read at 540 nm using spectrophotometer (SG8 072218, Spectronic GENESYS 8, England). Reducing sugar concentration was obtained from a standard glucose concentration curve.

β-1,4-Exoglucanase activity

The β-1,4-exoglucanase activity was assayed as above using microcrystalline cellulose (Avicel) as substrate.

β-Glucosidase activity

The β-glucosidase activity was assayed by incubating 0.1 ml of the culture filtrate with 0.5 ml of 0.05 M acetate buffer (pH 5.0) containing 2.5 mg cellobiose at 50°C for 10 min (Zaldivar et al., 2001). 10 µL of the glucose released was added to 1 ml glucose oxidase peroxidase reagent (Sigma) and allowed to stand for 10 min at room temperature before the optical density was read at 546 nm. The concentration of the glucose released (mg/ml) was measured as OD sample/ OD standard x concentration of standard.

Xylanase activity

Xylanase activity was determined by measuring the release of reducing sugars from a solution of water soluble birch wood xylan (Fluka BioChemika, 95588) using the dinitrosalicylic acid (DNS) method (Gawande and Kamat, 1999). The reaction mixtures containing 10 mg Xylan (Fluka BioChemika, 95588) in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml culture supernatant were incubated at 50°C for 30 min. The xylose formed was measured with dinitrosalicyclic acid (DNS).

Total extracellular protein

The total extracellular protein was determined by Lowry's method using bovine serum albumin (BSA) as standard (Lowry et al., 1951). Five milliliter (5 ml) of alkaline solution was added to the protein sample solution. This was mixed thoroughly and allowed to stand at room temperature for 10 min. Folin-Ciocalteau reagent (0.5 ml) was added and mixed. After 30 min, the absorbance was read against reagent blank at 750 nm. The protein concentration in the test sample was estimated from the standard protein concentration plot.

Table 1. Effect of carboxymethylcellulose on cellulase activity in fungal isolates.

Isolates	Total protein (µg/ml)	Enzyme Activity (U/ml)			
		Exoglucanase[a]	Endoglucanase[b]	β-glucosidase[c]	Xylanase[d]
Curvularia pallescens	195.00 ± 12.94	3.70 ± 0.43	0.95 ± 0.03	0.99 ± 0.04	2.32 ± 0.10
Fusarium solani	190.63 ± 16.25	2.87 ± 0.07	0.92 ± 0.03	0.60 ± 0.04	1.53 ± 0.02
Myrothecium roridum	130.00 ± 5.83	0.40 ± 0.02	0.36 ± 0.03	1.46 ± 0.32	0.78 ± 0.01

The cultures were grown at 120 rpm and 25 ± 2°C for 16 days. Values are Mean ± SEM of Triplicate Results from independent experiment. [a]Exoglucanase is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from microcrystalline cellulose per min. [b]Endoglucanase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from carboxymethyl cellulose per min. [c]One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1 µmole of glucose from cellobiose per min. [d]One unit of xylanase activity is defined as the amount of enzyme liberating 1 µmole of xylose from xylan per min.

Table 2. Effect of sawdust on cellulase activity in fungal isolates.

Isolates	Total protein (µg/ml)	Enzyme Activity (U/ml)			
		Exoglucanase[a]	Endoglucanase[b]	β-glucosidase[c]	Xylanase[d]
Curvularia pallescens	195.36 ± 9.82	2.35 ± 0.16	0.84 ± 0.04	1.41 ± 0.04	1.06 ± 0.03
Fusarium solani	270.00 ± 23.45	1.77 ± 0.17	0.48 ± 0.02	0.24 ± 0.02	1.58 ± 0.05
Myrothecium roridum	220.00 ± 13.96	0.43 ± 0.06	0.39 ± 0.02	1.74 ± 0.06	1.03 ± 0.01

The cultures were grown at 120 rpm and 25 ± 2°C for 16 days. Values are Mean ± SEM of Triplicate Results from independent experiment. [a]Exoglucanase is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from microcrystalline cellulose per min. [b]Endoglucanase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from carboxymethyl cellulose per min. [c]One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1 µmole of glucose from cellobiose per min. [d]One unit of xylanase activity is defined as the amount of enzyme liberating 1 µmole of xylose from xylan per min.

RESULTS

Determination of the cellulolytic activity of the isolates

The organisms used in this study were able to grow in the various carbon sources employed. This is an indication that cellulolytic enzymes were secreted by the isolates to depolymerize the carbon sources to simple sugars for growth. The result obtained showed that the fungi *C. pallescens* Boedjin, *F. solani* and *M. roridum* (IMI 394934) produced cellulase and xylanase activity during the fermentation period in submerged cultures (Tables 1 - 3).

Table 1 shows the enzyme production by the isolates in carboxymethylcellulose. The enzyme production was maximum with *C. pallescens* and minimum with *M. roridum*.

Similarly, *C. pallescens* and *M. roridum* produced the highest and lowest amount of cellulase enzyme respect-tively, in submerged culture containing sawdust as carbon source (Table 2). However, the enzyme activity by the organisms on sawdust was lower when compared to that produced in carboxymethylcellulose. A similar trend in enzyme production was also obtained when the isolates were grown in submerged culture containing water hyacinth as the carbon source. However, this medium gave the least amount of enzyme induction in the isolates (Table 3). The results in this study showed that the only pathogenic organism to water hyacinth, *M. roridum* (IMI 394934) is the poorest cellulase and xylanase enzyme producer (Tables 1 - 3). The results also indi-cates that carboxymethylcellulose (CMC) is the best carbon source in cellulase and xylanase enzyme induction in the isolates employed in this work (Tables 1 - 3).

DISCUSSION

Cellulase activity of phytopathogens

Literatures have shown that *Curvularia* sp. (Banerjee, 1990; Nitharwal et al., 1991; Banerjee and Chakrabarti, 1992), *F. solani* (Wood, 1971; Wood and McCrae, 1977; Gupta et al., 2009) and *Myrothecium* sp. (Singh and Shukla, 1985; Filho et al., 1994; Moreira et al., 2005) are capable of producing cellulase, β-glucosidase and xylanase enzyme in submerged cultures of lignocellulosic materials. However, in this present study, the enzyme production capacities of the three new strains of organisms (water hyacinth isolates) on three different carbon sources (carboxymethylcellulose, sawdust and water hyacinth) under the same cultural conditions were comparatively examined. Cellulase enzymes were produced by the three fungal isolates on the different substrates.

Table 3. Effect of water hyacinth on cellulase activity in fungal isolates.

Isolates	Total protein (µg/ml)	Enzyme Activity (U/ml)			
		Exoglucanase[a]	Endoglucanase[b]	β-glucosidase[c]	Xylanase[d]
Curvularia pallescens	225.39 ± 15.36	0.28 ± 0.01	0.12 ± 0.01	0.08 ± 0.00	0.30 ± 0.00
Fusarium solani	264.24 ± 12.23	0.32 ± 0.00	0.11 ± 0.00	0.13 ± 0.01	0.34 ± 0.01
Myrothecium roridum	324.00 ± 19.51	0.12 ± 0.02	0.13 ± 0.03	1.43 ± 0.02	0.34 ± 0.01

The cultures were grown at 120 rpm and 25 ± 2°C for 16 days. Values are Mean ± SEM of Triplicate Results from independent experiment. [a]Exoglucanase is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from microcrystalline cellulose per min. [b]Endoglucanase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from carboxymethyl cellulose per min. [c]One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1 µmole of glucose from cellobiose per min. [d]One unit of xylanase activity is defined as the amount of enzyme liberating 1 µmole of xylose from xylan per min.

The enzyme production was highest with *C. pallescens*, followed by *F. solani* and least with *M. roridum* (IMI 394934). The cellulase enzyme production by the three organisms was most favoured on the medium containing carboxymethylcellulose as the sole carbon source. This suggests that carboxymethylcellulose is a good carbon source for the induction of the enzyme in fungal species.

More so, studies have shown that cellulase production was higher upon growth of *Trichoderma harzianum* (Mes-Hartree et al., 1988), *Humicola fuscoatra* (Rajendran et al. 1994) and *A. niger* (Hanif et al., 2004) on cellulosic substrates.

Volvariella diplasia produced cellulolytic enzymes when grown in shake culture containing 0.5% cellulose powder (Puntambekar, 1995).

These observations are well in agreement with the results of the present study. It is therefore evident that the presence of cellulose of carboxymethylcellulose is responsible for the highest support for enzyme production by the isolates.

The use of carboxymethylcellulose for a large scale enzyme production may be uneconomical. One of the cheaply available agricultural lignocellulosic waste (saw-dust) used in this study also induced a favourable amount of enzymes in the organisms. The activities of β-glucosidase and xylanase were also appreciable in culture filtrate of the isolates grown on sawdust. This carbon source has been reported as a good inducer of cellulase in fungi, particularly when it is pretreated (Lo et al., 2005; Narasimha et al., 2006; Mohammed and Obasola, 2007; Milala et al., 2009).

In this study, the water hyacinth leaf appeared the poorest carbon source for enzyme induction in the three isolates. This suggests that the level of cellulose in the water hyacinth leaf was too small to induce enzyme synthesis or that there could be some enzyme inhibitors or proteinases in the water hyacinth leaf which represses the synthesis of cellulases and hemicellulases in the organisms. Plant proteinases have been implicated in the inhibiton of enzyme production by a plant pathogenic fungus (Moreira et al., 2005).

The production of β-glucosidase was highest by the water hyacinth pathogenic fungus when compared to the non-pathogenic isolates. The β-glucosidase enzyme production was highest when compared to the endoglu-canase, exoglunase and xylanase enzyme from the same pathogenic isolate. A related species, *Myrothecium verucarria* has been shown to produce a similar trend in result with β-glucosidase, endoglucanase, exoglunase and xylanase when different carbon sources were used (Moreira et al., 2005).

The enzymes produced by *M. roridum* (IMI 394934) may be seen as pathogenic in the penetration of the plant material rather than the virulence factor since the avirulent isolates were able to produce higher amount of these enzymes.

Finally, this study shows that the phytopathogenic strain of *M. roridum* is capable of inducing cellulases and xyla-nase enzyme in submerged cultures but to a lesser degree compared to *F. solani* and *C. pallescence* Boedjin. Many research works has been focused on the optimization of enzyme production in fungi due to the myriads and continued demand for biotechnological and industrial application of enzymes. Therefore, further studies will also involve the development of mutant strains of these organisms with enhanced production of lytic enzyme for lignocellulosic waste decomposition.

Conclusion

In this study, it has been shown that *M. roridum* was capable of producing cellulase and xylanase in submerged cultures containing different carbon sources. However, these enzymes were better induced in *F. solani* and *C. pallescence* Boedjin which were non phytopatho-genic when compared to water hyacinth.

REFERENCES

Acharya PB, Acharya DK, Modi HA (2008). Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate. Afr. J. Biotechnol. 7(22): 4147-4152.

Akiba S, Kimura Y, Yamamoto K, Kumagai H (1995). Purification and characterizationof a protease-resistant cellulase from *Aspergillus niger*. J. Ferment. Bioeng. 79(2): 125-130.

Baer D, Gudmestad NC (1995). *In vitro* cellulolytic activity of the plantpathogen *Clavibacter michiganensis* subsp. *sepedonicus.* Can. J. Microbiol. 877-888.

Banerjee UC (1990). Production of beta-glucosidase (cellobiase) by *Curvularia* sp. Lett. Appl. Microbiol. 10(5): 197-199.

Banerjee UC, Chakrabarti S (1992). Production and properties of carboxymethylcellulase 3 (endo-1,4-b-glucanase) from *Curvularia lunata.* World J. Microbiol. Biotech. 8(4): 423-424.

Christov LP, Szakacs G, Balakrishnan H (1999). Production, partial characterization and use of fungal cellulase-free xylanases in pulp bleaching. Process Biochem. 34: 511-517.

Filho EFX, Puls J, Coughlan MP (1994). Physicochemical and catalytic properties of a low molecular weight endo 1,4 β-xylanase from *Myrothecium verrucaria*. Enz. Microbiol. Technol. 15: 535-540.

Gielkens MMC, Dekkers E, Visser J, Graaff LH (1999). Two cellubiohydrolase-encoding genes from *Aspergillus niger* require DXylose and the xylanolytic transcriptional activator XlnR for their expression. Appl. Environ. Microbiol. 65(10): 4340-4345.

Gawande PV, Kamat MY (1999). Production of Aspergillus xylanase by lignocellulosic waste fermentation and its application. J. Appl. Microbiol. 87: 511-519.

Gupta VK, Gaur R, Gautam N, Kumar P, Yadav IJ, Darmwal NS (2009). Optimization of Xylanase Production from *Fusarium solani* F7. Am. J. Food Technol. 4(1): 20-29.

Han SJ, Yoo YJ, Kang HS (1995). Characterization of a bifunctional cellulase and Its structural gene. J. Biol. Chem. 270(43): 26012-26019.

Hanif A, Yasmeen A, Rajoka MI (2004). Induction, production, repression and de-repression of exoglucanase synthesis in Aspergillus niger. Bioresour. Technol. 94: 311-319.

Hong J, Tamaki H, Akiba S, Yamamoto K, Kumaga H (2001). Cloning of a gene encoding a highly stable endo-β-1,4-glucanase from *Aspergillus niger* and Its expression in Yeast. J. Biosci. Bioeng. 92(5): 434-441.

Ikram-ul-Haq, Javed MM, Siddiq Z, Saleem T (2006). Triggering of β-glucosidase Production in *Trichoderma viride* with Nutritional and Environmental Control. J. Appl. Sci. Res. 2(11): 884-889.

Jorgensen H, Kutter JP, Olsson L (2003). Separation and quantification of cellulases and hemicellulases by capillary electrophoresis. Analytical Biochemistry 317: 85–93.

Kang SW, Ko EH, Lee JS, Kim SW (1999). Over production of β-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. Biotechnol. Lett. 21:647-650.

Lee RL, Paul JW, Willem HV, Isak SP (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiology and Molecular Biology Reviews, 66(3): 506-577.

Lo CM, Zhang Q, Lee P, Ju LK (2005). Cellulase production by *Trichoderma reesei* using sawdust hydrolysate. Appl. Biochem. Biotechnol. 122(1-3): 561-573.

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

Mes-Hartree M, Hogan CM, Saddler JN (1988). Influence of growth substrate on production of cellulase enzymes by Trichoderma harzianum E-58. Biotechnol. Bioeng. 31: 725-729.

Milala MA, Shehu BB, Zanna H, Omosioda VO (2009). Degradation of Agro-Waste by Cellulase from *Aspergillus candidus*. Asian J. Biotechnol. 1(2): 51-56.

Mohammed IJ, Obasola EF (2007). Cellulase Production and Enzymatic Hydrolysis of Some Selected Local Lignocellulosic Substrates by a Strain of *Aspergillus niger.* Res. J. Biol. Sci. 2(1): 13-16.

Moreira FG, Simone R, Costa MAF, Marques de Souza CG, Peralta RM (2005) Production Of Hydrolytic Enzymes by the Plant Pathogenic Fungus *Myrothecium Verrucaria* In Submerged Cultures. Braz. J. Microbiol. 36: 7-11.

Murai T, Ueda M, Kavaguchi T, Arai M, Tanaka M (1998). Assimilation of cellooligosaccharides by a cell surface-engineered Yeast expressing β-glucosidase and carboxymethylcellulase from *Aspergillus aculeatus*. Appl. Environ. Microbiol. 64(12): 4857-4861.

Narasimha G, Sridevi A, Buddolla V, Subhosh CM, Rajasekhar RB (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. Afr. J. Biotechnol. 5(5): 472-476.

Nitharwal PD, Gour HN, Agarwal S (1991). Effects of different factors on the production of cellulase by *Curvularia lunata.* Folia Microbiol. 36: 357-361.

Okunowo WO, Gbenle GO, Osuntoki AA, Adekunle AA (2008a). Survey, evaluation and molecular characterization of Nigerian native fungus for potential biocontrol of water hyacinth. Phytopathology, 98: S115.

Okunowo WO, Gbenle GO, Osuntoki AA, Adekunle AA (2008b). Investigative study of *M. roridum* toxin on water hyacinth. Phytopathology, 98: S115.

Okunowo WO, Ogunkanmi LA (2009). Effects of sodium ion and water hyacinth extract in the production of *Curvularia pallescens* in culture media. Afr. J. Biochem. Res. 3(5): 238-244.

Parry JB, Stewart JC, Heptinstall J (1983). Purification of the major endoglucanase from *Aspergillus fumigatus* Freseius. Biochemistry, J. 213: 437-444.

Puntambekar US (1995). Cellulase production by the edible mushroom Volvariella diplasis. World J. Microbiol. Biotechnol. 11: 695.

Rajendran P, Gunasekharan P, Lakshmanan M (1994). Cellulase activity of Humicola fuscoatra. Indian J. Microbiol. 34: 289-295.

Riou C, Freyssinet G, Fevre M (1991). Production of cell wall-degrading enzymes by the phytopathogenic fungus *Sclerotinia sclerotiorum*. Appl. Environ. Microbiol. 57: 1478-1485.

Siddiqui KS, Shemsi AM, Anwar MA, Rashid MH, Rajoka MI (1999). Partial and complete alteration of surface charges of carboxymethylcellulase by chemical modification: thermostabilization in water-miscible organic solvent. Enzyme Microb. Technol. 24: 599-608.

Singh PN, Shukla P (1985). Production of pectolytic and cellulolytic enzymes by *Myrothecium roridum* causing leaf spot of cowpea. Indian J. Mycol. Plant Pathol. 13(3): 379-381.

Tarek AAM, Nagwa AT (2007). Optimization of cellulase and β-glucosidase induction by sugarbeet pathogen *Sclerotium rolfsii.* Afr. J. Biotechnol. 6(8): 1048-1054.

Teeri TT (1997). Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. Trends Biotechnol. 15: 160-167.

Viikari L, Kantelinen A, Sundquist J, Linko M (1994). Xylanases in bleaching from an idea to the industry. FEMS Microbiol. Rev. 13: 335-350.

Wood TM (1971). The cellulose of Fusarium solani, Purification and specificity of the β-(1-4)-glucanase and the β-D-glucosidase components. Biochem J. 121(3):353-62.

Wood TM, McCrae SI (1977). Cellulase from Fusarium solani: purification and properties of the C1 component. Carbohydr Res. 57: 117-33.

Zaldivar M, Velasquez JC, Contreras I, Perez LM (2001). Trichoderma aueviride 7-121, a mutant with enhanced production of lytic enzymes; its potential use in waste cellulose degradation and/or biocontrol. Elect. J. Biotechnol. pp. 1-7.