Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2011 Global Science Books

Kinetic Study and Characterization of 1,4-β-Endoglucanase of *Aspergillus niger* ANL301

Shalom Nwodo Chinedu[1*] • Obinna C. Nwinyi[1] • Uzoma A. Okafor[2] • Veronica I. Okochi[2]

[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, Lagos, Nigeria

Corresponding author: * **sncresearch@gmail.com** or **shchinedu@covenantuniversity.com**

ABSTRACT

Submerged fermentation of *Aspergillus niger* ANL 301 in basal medium containing cellulose as sole carbon source, yielded crude extracellular proteins with 0.54 ± 0.02 units mg protein^{-1} of 1,4-β-endoglucanase activity. Partial purification by ammonium sulphate precipitation (80% saturation) and gel filtration on Sephadex 25-300 gave two active fractions of 1,4-β-endoglucanase, which exhibited close activity towards carboxymethyl-cellulose (CMC). The pH profile of the pooled enzyme fractions showed three activity peaks at pH 3.5, 5.5 and 7.0. The enzyme was most active at pH 5.5 and showed optimal activity at 50°C. V_{max} of 4.4 ± 0.4 µmol min^{-1} mg protein^{-1} and K_m of 12.5 ± 0.4 gL^{-1} was obtained with CMC for the enzyme. Different divalent metal ions and EDTA affected the enzyme activity at 2.0 mM concentrations in different ways. Mn^{2+} and Fe^{2+} exhibited 253.4 and 24.0% stimulatory effects, respectively on the enzyme activity. Mg^{2+}, Ca^{2+}, Cu^{2+}, and Zn^{2+} inhibited the enzyme by between 22.3 and 29.4%, whereas 75.0 and 71.3% inhibition were obtained with Hg^{2+} and EDTA, respectively. Manganese ion showed an exceptional activation of the 1,4-β-endoglucanase. The organism produced two types of 1,4-β-endoglucanase with different molecular weights.

Keywords: *Aspergillus niger* ANL 301, carbon source, carboxymethyl-cellulose (CMC), 1,4-β-endoglucanase activity, kinetics, manganese ion

INTRODUCTION

Cellulase is the generic name for the family of enzymes which hydrolyze the β-glycosidic bonds of native cellulose and related cello-oligosaccharides (Chellapandi and Jani 2008). It is a key enzyme for the bioconversion of cellulosic biomass into simple sugars which can subsequently serve as feed-stock for the production of different chemicals and fuels via anaerobic fermentation (Zhang and Lynd 2004).

Filamentous microfungi are the most studied and best known cellulase-producing organisms because of their rapid growth, high enzyme yield and capacity to secrete active and separable cellulase enzyme types (Berry and Paterson 1990). Studies on hypersecretory microfungi such as *Trichoderma reesei*, *Penicillium pinophilum*, *Penicillium funiculosum*, *Fusarium oxysporum*, *Aspergillus niger*, *Sclerotium rolfsii* and *Humicola* sp. show that the enzyme is adaptive and also regulated by catabolite repression in most fungi (Reese and Levinson 1952; Berry and Paterson 1990; Howard *et al*. 2003). Cellulose and other substances such as sophorose, sawdust, and sugarcane pulp are known to stimulate the enzyme production (Mandels and Reese 1956; Nwodo-chinedu *et al*. 2007). Complete hydrolysis of native cellulose by most fungi requires the synergistic action of three types of cellulase, namely, 1,4-β-endoglucanase [EC 3.2.1.4], also known as 1,4-β-glucan glucanohydrolase, endo-(1,4)-D glucanase, endoglucanase, endocellulase or CMCase, 1,4-β-exoglucanase [EC 3.2.1.91], also called exo-(1,4)-D glucanase, cellobiohydrolase, exocellulase, microcrystalline cellulase or avicelase, and β-glucosidase [EC 3.2.1.21], also called cellobiase (Khan 1980; Bhat and Bhat 1997). 1,4-β-endoglucanase generally initiate cellulase action within the cellulose chain by cleaving at random the β-1,4-glucosidic bonds, commonly in the amorphous parts of the cellulose chain, thereby generating glucose and cello-oligosaccharides of various lengths, and consequently new chain ends.

Aspergillus niger group is noted for the secretion of extracellular enzymes which hydrolyze the β-glycosidic bonds of native cellulose and associated hemicelluloses (de Vries and Visser 2001). Several strains of the fungus have been isolated and studied to assess their potential for commercial cellulase production using different fermentation substrates and methods (Acharya *et al*. 2008; Chinedu *et al*. 2008a; Omojasola and Jilani 2008; Bjorn *et al*. 2009; Chinedu *et al*. 2010; Gamara *et al*. 2010). *Aspergillus niger* ANL301 was isolated from a wood-waste dump in Lagos, Nigeria (Nwodo-Chinedu *et al*. 2005). The organism grows effectively in basal medium supplemented with sawdust or sugarcane as sole carbon sources (Nwodo-Chinedu *et al*. 2007) and produces cellulases (Chinedu *et al*. 2008a) and xylanases (Okafor *et al*. 2007; Chinedu *et al*. 2008b) in media containing cellulose or agrowastes as sole carbon sources. In the present study, 1,4-β-endoglucanase of *A. niger* ANL301 was partially purified and characterized. The kinetic properties and effect of metal ions on the enzyme activity were investigated.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. Potato dextrose agar (PDA) and crystalline cellulose (Avicel) were obtained from Merck, Germany. Carboxymethyl-Cellulose (CM52) was obtained from Whatman Ltd., UK. All other chemicals and reagents were obtained from Sigma Chemicals Co. Ltd., UK.

Organism

The strain of *Aspergillus niger* ANL301 was isolated from wood-wastes in Lagos, Nigeria and identified as described previously

Received: 22 May, 2010. Accepted: 1 December, 2010.

Original Research Paper

(Nwodo-Chinedu *et al*. 2005). The organism was maintained at 4°C on PDA slants.

Cultivation and enzyme production

Fresh culture of the organism was made on sterile PDA plates and incubated at 30°C for 72 h. Colonies on the plates were covered with 10 mL of 0.1% (v/v) Tween 80. The spores were harvested using sterile cotton swab and transferred into a sterile test tube. The spore suspension served as inoculum for enzyme production. The extracellular enzymes were produced through submerged fermentation. The organism was grown on basal medium containing (per liter of distilled water): $NaNO_3$, 3.0 g; KCl, 0.5 g; KH_2PO_4, 1.0 g; $MnSO_4 \cdot 7H_2O_4$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and 10.0 g cellulose. One liter (1 L) of the media was supplemented with 1.0 mL of trace solution containing (per L of distilled water) $ZnSO_4$, 1.0 g and $CuSO_4 \cdot 5H_2O$, 0.5 g. The pH of each media was adjusted to 5.6. Conical flasks (250 mL) containing 100 mL of respective media were autoclaved at 121°C for 15 min, cooled and inoculated with 1.0 mL of spore suspension ($2–4 \times 10^6$ spores per mL) of the pure fungal isolate. The cultures were incubated for 72 h with continuous agitation at 100 rpm using a Griffin flask shaker. Cells were harvested by centrifugation at 6000 × *g* for 15 min at 4°C using an ultra centrifuge (Super-speed RC-B, USA). The cell-free culture supernatant was used as source of crude extracellular enzyme.

Protein assay

Protein content of the culture supernatant was determined by the Folin-Ciocalteau method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard. The protein concentration of the chromatographic fractions was determined by extinction at 280 nm wavelength with a spectrophotometer (Thermospectronic Genesys 4001/1, USA).

1,4-β-Endoglucanase assay

A modification of the reducing sugar method described by Khan (1980) was used to for the assay of 1,4-β-endoglucanase (EC 3.2.1.4) activity. Carboxymethyl-cellulose (CMC) was used as enzyme substrate. The reaction mixture contained 2.0 mL of 0.1% (w/v) CMC in 0.1M sodium acetate buffer (pH 5.0) and 2.0 mL of cell-free culture supernatant (or 0.5 mL of partially purified enzyme). The mixture was incubated at 40°C in a water bath with shaking for 30 min. The reducing sugar released was measured using 3, 5-dinitrosalicylic acid and read at 540 nm using a spectrophotometer (Miller 1959). The released reducing sugar was expressed in glucose equivalent and expressed in Units mL^{-1}. A unit of activity was defined as amount of enzyme required to liberate 1 µmol of glucose per minute under the assay conditions.

Partial purification of 1,4-β-endoglucanase

Partial purification of the crude extracellular enzyme was achieved by the process of freeze drying, ammonium sulphate precipitation/ dialysis, and gel chromatography. These were carried out in duplicates. Two hundred milliliters of the crude extracellular enzyme was freeze dried at - 4°C. The enzyme was redissolved in 20 mL acetate buffer (0.05 M, pH 5.0) and precipitated with $(NH_4)_2SO_4$ at 80% (w/v) saturation. This was centrifuged at 1000 × *g* for 15 min. The supernatant was carefully decanted and the precipitate was redissolved in the acetate buffer and dialyzed over-night at 4°C against the buffer.

Gel chromatography (molecular sieving) was done using Sephadex G 25-300 (Sigma). Ten-grams of Sephadex (Sigma) was suspended in 50 mL sodium acetate buffer (0.05 M, pH 5.0) and packed into a glass column (12 × 400 mm) at room temperature. The gel in the column was further washed with the same buffer. Five-mL of the concentrated dialyzed enzyme was then introduced to the top of the sephadex-packed column. After the enzyme had been absorbed, the same buffer was passed through the column at a constant flow rate of 1 mL min^{-1}. Five-mL fractions were collected and assayed for their protein and 1,4-β-endoglucanase activity. Enzymatically active fraction were pooled and concentrated by freeze drying.

Properties of 1, 4-β-endoglucanase

1. pH

The pH profile of the enzyme was determined by varying the pH of the reacting mixtures between 3.0 and 9.0. These substrates were prepared in two buffer solutions: 0.1 M acetate-NaOH (pH 3.0 to 7.0) and 0.1 M Tris-HCl buffer (pH 8.0 to 9.0).

2. Temperature

The temperature profile of the enzyme was determined by incubating the enzyme with substrates for 30 min at various temperatures between 30-80°C. The enzyme activity values were determined.

3. Time course

The time course of the enzyme was determined by measuring the enzyme activity at different period of incubation under the above standard assay conditions of pH 5.5 and 50°C. Total reducing sugars (glucose equivalent) released by the enzyme per time was monitored at 10-min intervals for 60 min.

4. Effect of substrate concentration

The effect of various substrate (CMC) concentrations (2.0 to 20 g/L) on the enzyme activity was studied under the above standard assay conditions of pH (5.0) and temperature (40°C). The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) of the enzyme were obtained using the reciprocal plot (Line-weaver–Burk plot).

5. Effect of metal ions and EDTA

The effect of divalent cations and ethylene diamino tetraacetic acid (EDTA) on the enzyme activity was determined by incubating the standard enzyme-substrate (CMC) mixture containing 2.0 mM salts of the respective cations at the standard assay conditions for 30 min. The salts included $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 5H_2O$, $CuSO_4 \cdot 7H_2O$, $CaSO_4$, $HgCl_2$, $FeSO_4 \cdot 7H_2O$. Effect of cation-chelator, EDTA, on the enzyme activity was also determined by including 10.0 mM of EDTA in enzyme-substrate mixture.

Data analysis

All assays were in triplicates, unless otherwise stated, and the results are expressed as Mean ± SEM (Standard Error of Mean).

RESULTS

Partial purification of 1,4-β-endoglucanase

Table 1 shows the purification steps for the 1,4-β-endoglucanases of *A. niger* ANL301. The enzyme was purified by 1.5-fold using ammonium sulphate at 80% saturation and 8.6-fold through gel filtration with Sephadex G25-300.

Table 1 Purification of 1, 4-β-endoglucanases of *Aspergillus niger* ANL301.

Purification step	Total activity (Unit)	Total protein (Mg)	Specific activity (Units Mg $protein^{-1}$)	Enzyme yield (%)	Purification fold
Crude enzyme	64.8 ± 2.4	120.0 ± 10.0	0.54 ± 0.02	100	1
$(NH_4)_2SO_4$	34.2 ± 1.8	42.6 ± 3.2	0.80 ± 0.03	52.8	1.5
Sephadex G25-300	23.7 ± 0.8	5.1 ± 0.2	4.65 ± 0.30	36.6	8.6

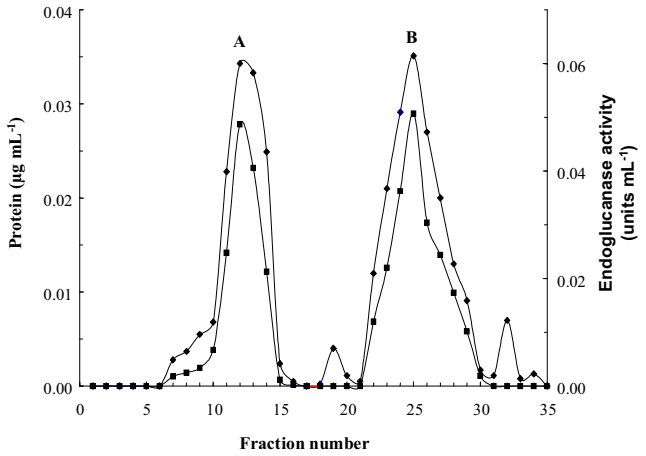

Fig. 1 Elution profile of 1, 4-β-endoglucanase of *A. niger* ANL301 on Sephadex G25-300. Graph shows protein content (♦), enzyme activity (■), and the two active enzyme fractions, (**A** and **B**). (0.1M Acetate buffer, pH 5.0; Flow rate = 1.0 mL min^{-1}).

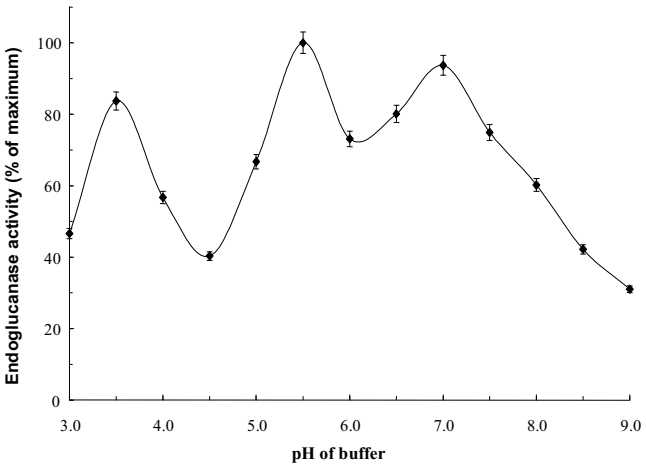

Fig. 2 Effect of different pH (3.0-9.0) on 1, 4-β-endoglucanase of *A. niger* ANL301. (100 % = 4.65 ± 0.30 unit mg $protein^{-1}$).

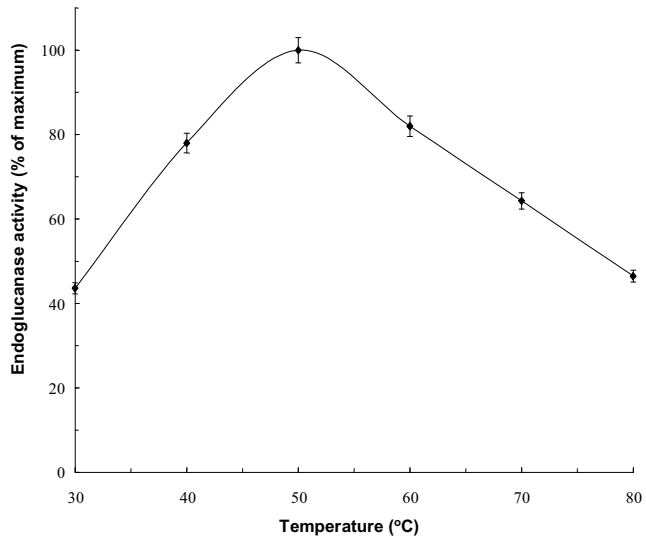

Fig. 3 Effect of different temperatures (30-80°C) on the 1, 4-β-endoglucanases of *A. niger* ANL301. (100 % = 4.65 ± 0.30 unit mg $protein^{-1}$).

The elution profile of the 1,4-β-endoglucanase is shown in **Fig. 1**. Two different active enzyme peaks were obtained. The 1,4-β-endoglucanase fractions A and B gave the activity of 0.049 ± 0.002 and 0.051 ± 0.002 units mL^{-1}, respectively.

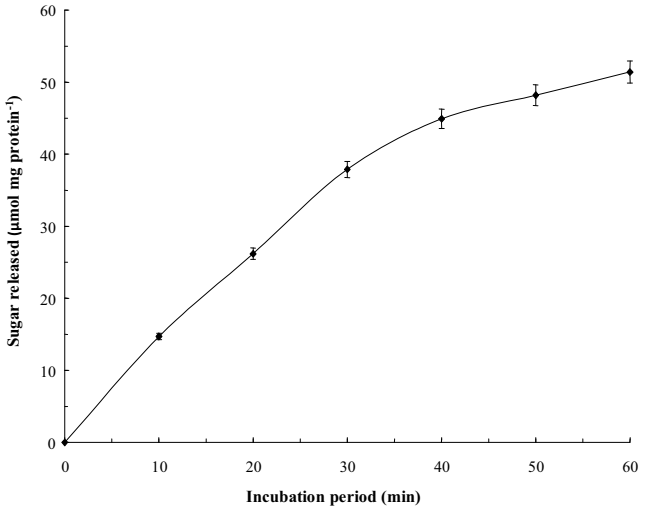

Fig. 4 Time course of 1, 4-β-endoglucanase of *A. niger* ANL301.

Properties of 1,4-β-endoglucanase

The effects of different pH, temperatures, metal ions and substrate concentrations on the partially purified 1,4-β-endoglucanases of *A. niger* ANL301 were determined.

Effect of pH

Fig. 2 shows the effect of different pH (3.0-9.0) on the activity of the 1,4-β-endoglucanases of *A. niger* ANL301. Three activity peaks (pH 3.5, 5.5 and 7.0), with the highest peak at pH 5.5, were obtained for the enzyme.

Temperature profile

The effects of different temperatures (30-80°C) on the 1,4-β-endoglucanases of *A. niger* ANL301 is shown in **Fig. 3**. The enzyme showed optimal activity at 50°C, with over 50% activity at 70°C.

Time course of 1, 4-β-endoglucanases

Fig. 4 shows the total reducing sugars released by the 1,4-β-endoglucanases of *A. niger* ANL301 at the different time of incubation. Total reducing sugars released increased with incubation time, but at disproportionate rates. There was a very rapid release of glucose in the first 30 min followed by a decline thereafter up to 60 min. The average rate of the release of glucose in the first 30 min was 1.25 µmol glucose mg $protein^{-1}$ min^{-1}.

Effect of substrate concentration on 1,4-β-endoglucanases activity

Plots of endoglucanase activities versus substrate concentrations showed normal hyperbola curve (**Fig. 5**). The cellulase activity increased rapidly as the substrate concentration increased between 2.0–10.0 gL^{-1}. Thereafter, subsequent increases in the substrate concentration had very small effect on the rate of enzyme activity. The Line Weaver-Burk plot (**Fig. 6**) was used to determine the maximum velocities (V_{max}) and Michaelis-Menten constants (K_m) of the enzyme. Maximum velocity (V_{max}) of 4.4 µmol min^{-1} mg $protein^{-1}$ was obtained for the endoglucanase *Aspergillus niger* The K_m value was 12.5 gL^{-1}.

Effects of metal ions and EDTA on the 1,4-β-endoglucanases activity

The effects of the metal ions and EDTA on 1,4-β-endoglucanase activity is shown in **Table 2**. Manganese (Mn^{2+}) and iron (Fe^{2+}) increased the enzyme activity by 253.4 and

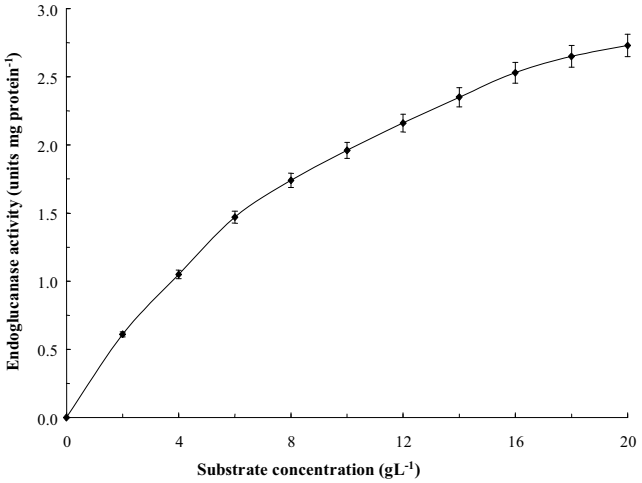

Fig. 5 Effect of substrate (CMC) concentration on the velocity (activity) of 1, 4-β-endoglucanase of *A. niger* ANL301.

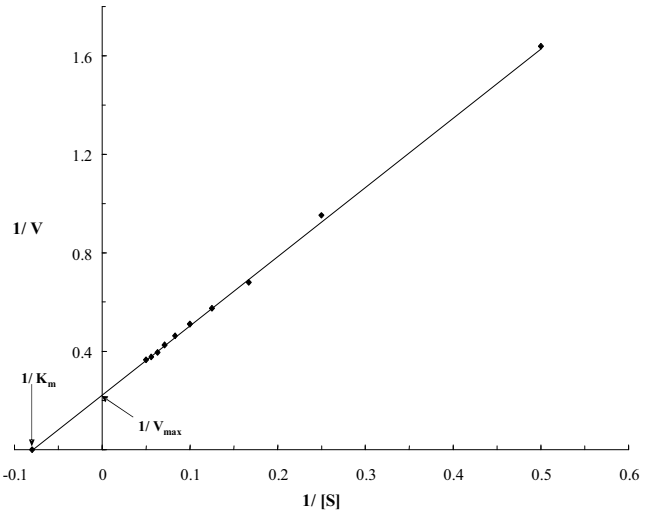

Fig. 6 Lineweaver-Burk plot (1/V versus 1/[S]) of the 1,4-β-endoglucanase of *A. niger* ANL301.

Table 2 Effects of metal ions and ethylene diamino tetraacetic acid (EDTA) on the activity of the cellulase enzyme of *Aspergillus niger* ANL 301.

Salts	Metal ions	Concentration (mM)	% Activity	% Inhibition	% Stimulation
Control (None)	-	-	100	-	-
$MgSO_4.7H_2O$	Mg^{2+}	2.0	77.7	22.3	-
$MnSO_4.5H_2O$	Mn^{2+}	2.0	353.4	-	253.4
$FeSO_4.7H_2O$	Fe^{2+}	2.0	124.0	-	24.0
$CaCl_2$	Ca^{2+}	2.0	74.3	25.7	-
$CuSO_4.7H_2O$	Cu^{2+}	2.0	72.0	28.0	-
$ZnSO_4$	Zn^{2+}	2.0	70.6	29.4	-
$HgCl_2$	Hg^{2+}	2.0	25.0	75.0	-
EDTA	-	10.0	28.7	71.3	-

24.0%, respectively. EDTA and some metal ions inhibited the enzyme activity. The percentage inhibition on the enzyme activity were 22.3, 25.7, 28.0, 29.4, 75.0 and 71.3% for Mg^{2+}, Ca^{2+}, Cu^{2+}, and Zn^{2+}, Hg^{2+} and EDTA, respectively.

DISCUSSION

Cellulase is recognized as a potential tool for the industrial saccharification of cellulosic biomass; its production is regarded as crucial for the successful utilization of cellulosic materials (Wu and Lee 1997; Solomon *et al*. 1999; Zhang and Lynd 2004). The expanding uses of cellulase and need for its viable commercial production have continued to propel the search for new strains of cellulase-producing organisms as well as cost-effective means for the enzyme production (Coral *et al.* 2002; Ojumu *et al*. 2003). *Aspergillus* species are major sources of commercial cellulases (Berry and Paterson 1990). The specific cellulase activity of 0.54 units mg protein^{-1} (97.2 µg glucose min^{-1} mg protein^{-1}) obtained for the crude extracellular enzyme preparation of *A. niger* ANL301 compared favorably with the activity of 109.0 µg glucose min^{-1} mg protein^{-1} reported for a commercial cellulase preparation from a strain of *A. niger* (Khan 1980). 1,4-β-Endoglucanase is the major component of cellulase from *A. niger* (Khademi *et al.* 2002). The production of 1,4-β-endoglucanases and xylanases (Okafor *et al*. 2007; Chinedu *et al.* 2008b) by this strain of *A. niger* is indicative of its potential as a source of enzymes for industrial saccharification of cellulosic biomass. Xylanase is also required for the hydrolysis of native cellulose (Khan 1980).

The endoglucanase of *A. niger* (ANL301) showed two different activity peaks on Sephadex G 25-300 gel chromatography. Gel chromatography separates proteins according to their molecular weights. The two fractions therefore imply the organism produces two different types of endoglucanases which differ in their molecular weights. Most cellulolytic fungi are known to produce isoenzymic forms of the endoglucanase having different molecular weights (Beldman *et al.* 1985; Berry and Paterson 1990). For instance, the *Trichoderma reesei* cellulase mixture consists of many catalytically active proteins; at least five endoglucanases (EG 1–5), two cellobiohydrolases (CBH 1-2), β-glucosidases, and hemicellulases have been identified by 2-dimentional electrophoresis (Beldman *et al*. 1985). Coral *et al*. (2002) obtained two protein bands with molecular weights of about 83,000 and 50,000 Da for the carboxymethyl-cellulase enzyme of a wild-type strain of *A. niger* Z10. Two protein bands have also been obtained respectively for the crude enzymes of *A. niger* and *A. fumigatus* with SDS-polyacrylamide gel electrophoresis (Immanuel *et al.* 2007). Hasper *et al.* (2002) reported the isolation of a novel endoglucanase, EglC, which is most active towards xyloglucan and functionally different from two other endoglucanases, EglA and EglB, from *A. niger.* The two enzyme fractions obtained in this study may therefore represent two isoenzymic forms of endoglucanase produced by *A. niger* (ANL301).

The optimum temperature of the enzyme was 50°C; it also retained over 50% of its activity at 70°C under the assay condition. This may be as a result of adaptation to the hot-humid climate from where the organisms were obtained. Coral *et al*. (2002) reported an optimal temperature of 40°C for the carboxymethyl-cellulase enzyme of a wild-type strain of *A. niger* Z10. The higher optimal temperature obtained for the endoglucanase of the organism implies better heat stability. This is desirable, especially in industrial processes where thermal treatment may be inevitable. An optimum temperature of 50°C has been reported for the endoglucanase from *A. glaucus* XC9 (Tao *et al*. 2010). Some commercial cellulase enzymes have also been reported to be stable at 60°C (Immanuel *et al*. 2007).

The cellulase enzyme of *A. niger* ANL301 has a broad pH range of between 3.5 and 7.5 with three major activity peaks at 3.5, 5.5 and 7.0. A broad pH range between 3 to 9 was reported for a wild type strain of *A. niger* Z10 (Coral *et al.* 2002). This is probably due to the isoenzymes or sub-

units of the endoglucanase enzyme. The implication is that the endoglucanase can tolerate variations in pH and function effectively at both acidic and neutral environments. With an optimum pH of the enzyme is 5.5, the cellulase enzyme is well suited for the acidic environment of most fermentation processes, especially for simultaneous saccharification and fermentation (SSF) of lignocelluloses. The SSF of sawdust and sugarcane pulp by *A. niger* ANL301 and the brewer's yeast, *Saccharomyces cerevisae*, carried out in our laboratory using yielded appreciable amounts of ethanol (unpublished data).

A hyperbolic curve was obtained for the activities of the cellulase enzyme. The Line Weaver-Burk plot gave a V_{max} of 4.4 µmolmin^{-1} mg protein^{-1} and K_m value of 12.5 g/ L. The V_{max} of the endoglucanase of *A. niger* was less than 10.0 µmolmin^{-1} mg protein^{-1} obtained for the endoglucanase of *P. chrysogenum* PCL 501 in our laboratory.

Metal ions and EDTA were found to have profound effects on the activity of the endoglucanases of the organism. Manganese (Mn^{2+}) and iron (Fe^{2+}) increased the enzyme activity by 253.4% and 24.0%, respectively. The endoglucanase activity of *A. glaucus* XC9 was reported to be stimulated by Mn^{2+} and Fe^{2+} (Tao *et al.* 2010). However, the stimulatory effect of manganese ions on the endoglucanase of *A. niger* ANL301 is quite phenomenal. The metal ion exhibited 253.4% stimulation of the activity of cellulase enzymes of the organism. This implies a significant increase in the yield of hydrolytic products. Fukumoto and Kishi (1952) had reported a phenomenal increase of cellulase activity by manganese stimulation, but their finding was largely ignored because, as at the time, no cellulase enzyme had been shown to have any prosthetic group or coenzyme, or to require any metal ions for its activity (Mandels and Reese 1956). Fahraeus (1947) in an earlier work, observed that *Cytophaga* cannot grow on cotton wool unless calcium and manganese were added to the medium. Manganese ion was shown to exert considerable stimulatory effect on 1,4-β-endoglucanase of the fungus, *Penicillium chrysogemum* PCL501 (Chinedu *et al.* 2008c). This work clearly indicates that manganese certainly has stimulatory effect on the activity of the endoglucanase of *A. niger* ANL301. There is the possibility that the manganese ion may indeed be a requirement for the enzyme activity and may even be an integral component of the enzyme complex. The potent inhibitory effect of EDTA, a chelating agent of divalent metal ions, on the enzyme activity may be connected with the removal of the manganese ions from the enzyme mixture. There is the need to further examine and establish the mechanism of manganese stimulation of the cellulase enzymes. Singh *et al*. (1990) from their studies on the kinetic properties of endoglucanase of *A. niger* AS-101 concluded that the enzyme was a metallo-protein or it required certain metal ions for activation. There is need for further investigations. Mercury ions on the other hand strongly inhibited the enzyme activity. This is normal since heavy metals are known to inhibit the activity of most enzymes. The activity of endoglucanase from *A. glaucus* XC9 was reported to be inhibited by the heavy metals, Cd^{2+}, Pb^{2+} and Cu^{2+} (Tao *et al.* 2010).

CONCLUSIONS

A. niger ANL301 produces extracellular proteins with cellulase activity comparable to commercially available cellulase preparation. Our data shows that the organism produces two types of 1,4-β-endoglucanase (EC 3.2.1.4) which differs in their molecular weight; they may be two isoenzymic forms of the enzyme. Manganese ion has been found to be an effective activator of the 1,4-β-endoglucanases of *A. niger* ANL301, with over 3-fold stimulatory effect on the hydrolytic activity of the enzyme. Further work is required to establish the mechanisms by which manganese ions stimulate the 1,4-β-endoglucanase of *A. niger* ANL301. The metal ion can be used to improve cellulase activity for industrial purposes.

REFERENCES

Acharya PB, Acharya DK, Modi HA (2008) Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate. *African Journal of Biotechnology* **7 (22)**, 4147-4152

Beldman G, Searle-van Leeuwen M, Rombouts F, Voragen F (1985) The Cellulose of *Trichoderma viride*: purification, characterization and comparison of all detectable endoglucanases, exoglucanases and beta-glucosidases. *European Journal of Biochemistry* **146**, 301-308

Berry DR, Paterson A (1990) Enzymes in food industry. In: Suckling CJ (Ed) *Enzyme Chemistry, Impact and Applications* (2nd Ed), Chapman and Hall, London, pp 306-351

Bhat MK, Bhat S (1997) Cellulose degrading enzymes and their potential industrial applications. *Biotechnology Advances* **15**, 583-620

Bjorn A, Shaunita HR, Willem HvZ, Anders S, Nils-Olof N, Leif JJ (2009) Cellulase production from spent lignocellulose hydrolysates by recombinant *Aspergillus niger*. *Applied and Environmental Microbiology* **75 (8)**, 2366-2374

Chellapandi P, Jani HM (2008) Production of endoglucanase by the native strains of *Strptomyces* isolates in submerged fermentation. *Brazillian Journal of Microbiology* **39**, 122-127

Chinedu SN, Eni AO, Adeniyi AI, Ayangbemi JA (2010) Assessment of growth and cellulase production of wild-type microfungi isolated from Ota, Nigeria. *Asian Journal of Plant Science* **9 (3)**, 118-125

Chinedu SN, Nwinyi OC, Okochi VI (2008a) Growth and cellulase activity of wild-type *Aspergillus niger* ANL301 in different carbon sources. *Canadian Journal of Pure and Applied Sciences* **2 (2)**, 357-362

Chinedu SN, Okafor UA, Emezue TN, Okochi VI (2008b) Xylanase production of *Aspergillus niger* and *Penicillium chrysogenum* from ammonia pretreated cellulosic waste. *Research Journal of Microbiology* **3 (4)**, 246-253

Chinedu SN, Nwinyi OC, Okochi VI (2008c) Properties of endoglucanase of *Penicillium chrysogemum* PCL501. *Australian Journal of Basic and Applied Sciences* **2 (3)**, 738-746

Coral G, Arikan B, Unaldi MN, Guvenmes H (2002) Some properties of crude carboxymethylcellulase of *Aspergillus niger* Z10 wild-type strain. *Turkish Journal Biology* **26**, 209-213

De Vries RP, Visser J (2001) *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Review* **65**, 497-552

Fahraeus G (1947) Studies in the cellulose decomposition by *Cytophaga*. *Symbolae Botan. Upsaliensis* **9**, 1-128

Fukumoto J, Kishi K (1952) Decomposing of cellulose by wood decaying microbes II. Purification of *Irpex* cellulase and its activity as affected by manganese. *Science et Industrie* **26**, 295-300

Hasper AA, Dekkers E, Mil MV, Vondervoort PJIVD, Graaff LHD (2002) EglC, a new endoglucanase from *Aspergillus niger* with major activity towards xyloglucan. *Applied and Environmental Microbiology* **68 (4)**, 1556-1560

Howard RLI, Abotsi E, Jansen Van Rensburg ELI, Howard S (2003) Lignocellulose biotechnology: Issues of bioconversion and enzyme production. Review. *African Journal of Biotechnology* **2 (12)**, 602-619

Immanuel G, Akila-Bhagavath CM, Iyappa-Raj P, Esakkiraj P, Palavesam A (2007) Production and partial purification of cellulase by *Aspergillus niger* and *A. fumigatus* fermented in coir waste and sawdust. *The Internet Journal of Microbiology* **3 (1)**, 1-20

Khademi S, Zhang D, Swanson SM, Wartenberg A, Witte K, Meyer EF (2002) Determination of the structure of an endoglucanase from *Aspergillus niger* and its mode of inhibition by palladium chloride. *Acta Crystallographica Section D*, **58**, 660-667

Khan AW (1980) Cellulolytic enzyme system of *Activibrio cellulolyticus*, a newly isolated Anaerobe. *Journal of General Microbiology* **121**, 499-502

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry* **193**, 265-275

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

Miller GL (1959) Use of dinitrosalicyclic reagent for the determination of reducing sugars. *Analytical Chemistry* **31**, 426-428

Nwodo-Chinedu S, Okochi VI, Smith HA, Omidiji O (2005) Isolation of cellulolytic microfungi involved in wood-waste decomposition: Prospect for enzymatic hydrolysis of cellulosic wastes. *International Journal of Biomedical and Health Sciences* **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. *African Journal of Biotechnology* **6 (3)**, 243-246

Ojumu TV, Solomon BO Betiku E, Layokun SK, Amigun B (2003) Cellulase production by *Aspergillus flavus* Linn Isolate NSPR101 fermented in sawdust, bagasse and corncob. *African Journal of Biotechnology* **2 (6)**, 150-152

Okafor UA, Okochi VI, Onyegeme-Okerenta MB, Nwodo-Chinedu S (2007) Xylanase production by *Aspergillus niger* ANL301using agro-wastes. *African Journal of Biotechnology* **6 (14)**, 1710-1714

Omojasola PF, Jilani OP (2008) Cellulase production by *Trichoderma longi*,

Aspergillus niger and *Saccharomyces cerevisae* cultured on waste materials from orange. *Pakistan Journal of Biological Sciences* **11 (20)**, 2382-2388

Reese ET, Levinson HS (1952) A comparative study of the breakdown of cellulose by microorganisms. *Physiologia Plantarum* **5**, 345-366

Ryu DD, Mandels M (1980) Cellulases: Biosynthesis and applications. *Enzyme Microbiology and Technology* **2**, 92-102

Singh A, Agrawal AK, Abidi AB, Darmwal NS (1990) General and kinetic properties of endoglucanase from *Aspergillus niger. FEMS Microbiology Letters* **71 (1-2)**, 221-224

Solomon BO, Amigun B, Betiku E, Ojumu TV, Layokun SK (1999) Optimization of cellulase production by *Aspergillus flavus* Linn isolate NSPR101 grown on bagasse. *Journal* of the *Nigerian Society of Chemical Engineers* **16**, 61-68

Tao Y-M, Zhu X-Z, Huang J-Z, Ma S-J, Wu X-B, Long M-N, Chen Q-X (2010) Purification and properties of endoglucanase from a sugar cane bagasse hydrolyzing strain, *Aspergillus glaucus* XC9. *Journal of Agriculture and Food Chemistry* **58 (10)**, 6126-6130

Wood TM, McCrae SI (1986) The cellulase of *Penicilium Pinophilum*. Synergism between enzyme components in solubilizing cellulose with special reference to the involvement of two immunologically distinct cellobiohydrolase. *Biochemistry Journal* **234**, 93-99

Wu Z, Lee YY (1997) Inhibition of the enzymatic hydrolysis of cellulose by ethanol. *Biotechnology Letters* **19**, 977-979

Zhang Y-HP, Lynd LR (2004) Towards an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and Bioengineering* **88**, 797-823