# Purification and Characterization of beta 1,4-Glucanases from *Penicillium simplicissimum* H-11

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In this study, β-1, 4-endoglucanase from *Penicillium simplicissimum* H-11 was purified to homogeneity using ammonium sulfate followed by Sephadex G-100 chromatography. The purity of the enzyme was confirmed by HPLC and 12% SDS-PAGE, indicating a single peak with a molecular mass of 33.2 kDa. This protein had mostly α-helix structures, as confirmed by FTIR spectrometry. The optimum pH and temperature were 3.2 and 60 °C with pH stability of 2.8~5.6 and temperature stability of 50 °C for 12 h and 4 h, respectively. A metal profile of the enzyme showed that $Mg^{2+}$ and $Sn^{2+}$ were strong activators, while $Cu^{2+}$ was a strong inhibitor. An interesting feature of this enzyme is that it can effectively hydrolyze microcrystalline cellulose, filter paper, and CMC-Na, thus revealing both endo- and exo-glucanase features of the enzyme. The kinetic constants $K_m$ and $V_{max}$ were 14.881 mg/mL and 0.364 mg/mL/min, respectively, against CMC-Na as a substrate.

*Keywords: CMCase; Penicillium simplicissimum; Purification; Characterization*

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## INTRODUCTION

Cellulose is the most abundant form of carbohydrate found in nature. It comprises 35 to 50% of the biomaterials in the world (Ragauskas *et al.* 2006) with annual production of $10^{10}$ tonnes/year (Singh and Hayashi 1995). It is mostly found in plant cell walls and is also produced by some animals, like tunicates and some bacteria (Lynd *et al.* 2002). Cellulose is a homopolysaccharide that consists of glucose units linked together via β1-4 glycosidic bonds, forming an unbranched linear structure. Cellulose molecules vary in length and are arranged in the form of bundles or fibrils (Walsh 2002). In these bundles, cellulose molecules are present in crystalline or amorphous forms (Walter 1998).

Cellulose is degraded by cellulase enzyme complexes, which consist of three types of enzymes: 1) endoglucanase (endo-1, 4-β-D-glucanase, EC3.2.1.4), 2) cellobiohydrolase (exo-1,4-β-D-glucanase, EC 3.2.1.91), and 3) β-glucosidase (1,4-β-D-glucosidase, EC 3.2.1.21) (Hong *et al.* 2001; Li *et al.* 2006). All three enzymes act synergistically on cellulose and convert it into glucose (Almin *et al.* 1975; Bucht and Ericksson 1969). These enzymes are produced by bacteria and filamentous fungi; however, the use of filamentous fungi is preferred due to ease and cost-effectiveness of production (Wood and McCrae 1982; Oliveira 2006).

The present work focuses on endoglucanases, which are produced by a wide variety of microorganisms, such as bacteria, fungi, yeasts, actinomycetes, algae, and myxobacteria (Tengerdy and Szakacs 2003; Krishna 2005). These enzymes can be produced through submerged fermentation and solid-state fermentation. Solid-state fermentation has advantages over submerged fermentation with respect to higher product stability and higher yields (Singhania *et al.* 2009; Holker and Lenz 2005). Submerged fermentation can be easily handled, compared to solid-state fermentation (Holker and Lenz 2005) because solid-state fermentation needs a longer lag time, large inoculum size, and different optimal growth conditions (Gowthamana *et al.* 2001). In solid-state fermentation, control of heat transfer is also a major problem in large-scale fermentation (Raghavarao *et al.* 2003).

Cellulase is particularly interesting due to its potential for application in various fields. These enzymes are widely used in animal feed production, starch processing, extraction of fruit and vegetable juices, grain alcohol fermentation, malting and brewing, the pulp and paper industry, saccharification of agriculture wastes for bioethanol technology, the textile industry, and the detergent industry (Ögel *et al.* 2001; Abo-State *et al.* 2010; Camassola and Dillon 2009; Vu *et al*. 2011). There are a many reports about cellulases of genus *Penicillium*, but this is the first report on endoglucanases from *Penicillium simplicissimum.* This study aimed to produce, purify, and characterize endo-glucanase from this species of fungus in submerged fermentation.

## EXPERIMENTAL

### Microorganism

A strain of *Penicillium simplicissimum* H-11 was obtained from the Biological Engineering Research Laboratory, Center of Life Science and Technology, Harbin Institute of Technology (Harbin, China). The strain was grown on PDA slants and used for CMCase enzyme production.

### Inoculum Development

Inoculum was developed using the following medium: 3.0 g/L $(NH_4)_2SO_4$, 0.005 g/L $FeSO_4 \cdot 7H_2O$, 1.0 g/L $KH_2PO_4$, 0.0016 g/L $MnSO_4 \cdot H_2O$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.0017 g/L $ZnSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2$, 0.002 g/L $CoCl_2$, 0.1 g/L NaCl, and 20 g/L ball-milled rice straw. This medium was inoculated with spores of five-day-old *Penicillium simplicissimum* and incubated at 30 °C for three days of fermentation with an agitation speed of 280 rpm. After termination of the fermentation period, this culture broth was used as an inoculum source.

### Enzyme Production

The medium used for enzyme production was comprised of: 18 g/L wheat bran, 13.5 g/L rice straw, 4.5 g/L bean cake powder, 0.4 g/L $KH_2PO_4$, 0.03 g/L $CaCl_2 \cdot 2H_2O$, and 0.03 g/L $MgSO_4 \cdot 7H_2O$. This medium was aseptically inoculated with a culture of *Penicillium simplicissimum.* Fermentation was carried out in a 20 L fermentation tank at 30 °C with an agitation speed of 280 rpm for four days of fermentation. After the end of the fermentation period, the fermentation broth was collected, filtered with gauze, and centrifuged at 8000 x g for 15 min at 4 °C. The cell free supernatant obtained after centrifugation was used as a source of crude CMCase enzyme.

## Assay of CMCase

CMCase activity was estimated as reported in the literature (Irfan *et al.* 2011) with a slight modification. The reaction mixture consisted of 0.5 mL substrate (1% CMC prepared in 0.05M citrate buffer pH 4.8), and 0.5 mL of enzyme solution was incubated at 50 °C for 30 min. After incubation, the reaction was stopped by the addition of 1.5 mL of DNS and then boiled for 10 min in boiling water bath. The reaction mixture was allowed to cool, and the reducing sugars released were measured spectrophotometrically at 540 nm. One unit of activity (U) was defined as the amount of the enzyme that liberated 1 µg sugar from the substrate per minute under standard assay conditions. Protein content in the filtrate was determined by Bradford's (1976) method using BSA as a standard.

## Purification of CMCase

The cell-free supernatant was precipitated by adding ammonium sulphate at different saturation levels (30 to 90%). After each addition, the enzyme solution was stirred for 1 h at 4 °C. The precipitated protein was collected by centrifugation at 8000 x g for 15 min at 4 °C and re-suspended in a minimum volume of 0.05 M citrate buffer (pH 4.8) to obtain the concentrated enzyme suspension. After that, the enzyme suspension was dialyzed with the same buffer using 3 to 5 changes of fresh buffer. The concentrated enzyme sample was purified on a Sephadex G-100 column (2 cm × 120 cm). The Sephadex column was equilibrated with 0.05 M citrate buffer (pH 4.8), and the dialyzed enzyme sample was loaded onto the Sephadex G-100 column eluted with the same buffer.

Fractions (5 mL/tube) were collected at a flow rate of 30 mL/h with a fraction collector. The fractions showing absorbance at 280 nm were analyzed for CMCase activity. The active fractions were pooled, dialyzed, and then lyophilized. The lyophilized enzyme sample was stored at -20 °C for further study.

SDS-PAGE (12%) was performed according to the method described by Laemmli (1970) using a mini slab gel apparatus.

The molecular weight was determined by interpolation from the linear semi-logarithmic plot of relative molecular weight *versus* the $R_f$ value (relative mobility) using standard molecular weight markers (low molecular weight markers, Pharmacia).

## HPLC analysis of CMCase

A Hypersil ODS column (4.6 mm × 100 mm) for high performance liquid chromatography (Agilent 1100 Series) was used to test the enzyme purity. The 5 µL sample volume was injected and separated using a solvent system of acetonitrile-water (70:30) at a flow rate of 1.0 mL/min. A highly sensitive MWD UV detector was used to read the absorbance.

## FTIR Analysis of CMCase

A mixture of sample and KBr (5% sample, 95% KBr) was passed into a disk for Fourier transform infrared spectroscopy (Magna-IR 560 ESP, FTIR Nicolet Company, USA) measurement. The spectra were recorded with 32 scans in the frequency range of 4000 to 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Disks were prepared in triplicate to obtain a constant spectrum.

### UV Absorption Spectrum of CMCase

The ultraviolet absorption spectrum of enzyme was recorded in an aqueous solution with a double beam spectrophotometer (VARIAN Cary 4000 UV-VIS spectrophotometer, USA) at ambient temperature (30 °C).

### Characterization of Enzyme

*Effect of temperature on activity and stability of CMCase*

The effect of temperature on the activity of CMCase was determined by incubating crude enzyme mixture in 1% CMC in 10 mM citrate buffer (pH 5.0) at temperatures between 40 to 90 °C with a regular interval of 5 °C. Enzyme activity was assayed by the DNS method at different temperatures, as described above. Thermo-stability studies of the enzyme were conducted by pre-incubating the enzyme solution at 30, 40, 50, 60, 70, and 80 °C for 4 h. After incubation, the enzyme activity was checked by the DNS method.

*Effect of pH on activity and stability of CMCase*

The optimum pH for the enzyme was determined by incubating the enzyme with substrate (1% CMC), prepared in a 0.05 M citrate buffer (pH 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, and 6.8). To check the stability at different pH, the enzyme was placed in different pH buffers at room temperature (30 °C) for 12 h. After, the enzyme activity was measured using a standard assay procedure.

*Effect of various metal ions on activity of CMCase*

Various metal ions, including $Sn^{2+}$, $Cu^{2+}$, $Li^{+}$, $Zn^{2+}$, $Co^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$, and $Mg^{2+}$, were applied to obtain the optimum activity of the enzyme. Each metal ion was used at a concentration of 10 mM.

*Substrate specificity*

Effects of various substrates, such as Xinghua filter paper, microcrystalline cellulose (1%), xylan (1%), CMC-Na (1%), pNPG (1%), pNPC (1%), chitin (1%), and salicin (0.5%) on purified CMCase activity were determined. The CMCase enzyme activity towards CMC-Na was taken as control.

*Enzyme kinetics*

The $K_m$ and $V_{max}$ of CMCase were calculated by linear regression analysis by a Lineweaver-Burk plot (double reciprocal plot) using various concentrations of CMC (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/mL). The experiments were carried out in triplicate, and the activity was measured according to standard assay conditions.

## RESULTS AND DISCUSSION

### Purification of CMCase

The enzyme CMCase was produced from *Penicillium simplicissimum* by submerged fermentation at 30 °C for a 72 h fermentation period. Table 1 summarizes the purification steps of the CMCase enzyme. The crude enzyme solution was fractionated by ammonium sulphate fractionation. After 80% ammonium sulphate saturation, the enzyme suspension was dialyzed using citrate buffer (pH 4.8) for 48 h at 4 °C. The

dialyzed enzyme solution was loaded onto a Sephadex G-100 column. The elution profile of the enzyme solution is shown in Fig. 1. The elution profile showed that there was only one peak. Fractions 9 to 19 from this peak were tested for CMCase activity. The fractions 11 to 17 exhibited higher specific activity (1577.94 U/mg) than the other fractions. A purification fold of 2.27 with yield of 33.2% was obtained from these fractions. From this step, specific activity improved from 695.23 U/mg to 1577.94 U/mg, indicating purification of the enzyme by Sephadex G-100 chromatography. Thus, these fractions (11 to 17) were pooled, dialyzed, and lyophilized. These findings were in close agreement with other reports that purified endoglucanase by Sephadex G-100 chromatography and obtained purification folds in the range of 2.09 to 3.43 (Pham *et al.* 2012; Iqbal *et al.* 2011; El-Zawahry *et al.* 2010). Chinedu *et al.* (2011) also purified an endoglucanase enzyme from *A. niger* NL301 and obtained an enzyme yield of 36.6% with a purification fold of 8.6 using Sephadex G25-300 chromatography. Singh *et al.* (2011) purified extracellular endoglucanase from *A. awamori* F18 and obtained 12-fold purification with ammonium sulphate fractionation followed by DEAE ion exchange chromatography.

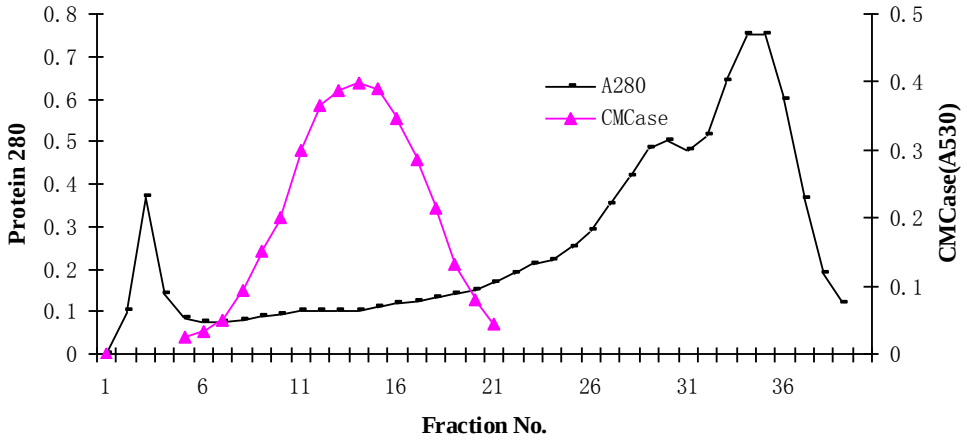


**Fig. 1.** Sephadex-G-100 elution profile of CMCase produced from *P. simplicissimum* in submerged fermentation at 30°C

**Table 1.** Purification Profile of CMCase Produced from *P. simplicissimum*

| Purification step | Total activity (IU) | Total protein (mg/mL) | Specific activity ($U \cdot mg^{-1}$) | Purification fold | Yield (%) |
|---|---|---|---|---|---|
| Crude enzyme | 1635.18 | 2.352 | 695.23 | 1.00 | 100.0 |
| Amm. sulphate ppt. | 902.5 | 1.06 | 851.4 | 1.22 | 55.2 |
| Sephadex G-100 | 542.81 | 0.344 | 1577.94 | 2.27 | 33.2 |

## Purity Check of CMCase

The enzyme solution obtained from Sephadex G-100 column chromatography was converted into powder form by lyophilization. The results of SDS-PAGE (Fig. 2) indicated a single band, which confirmed the homogeneity of the enzyme preparation. The molecular weight of the CMCase enzyme was determined by plotting a graph of linear logarithms of the relative molecular mass *versus* the $R_f$ value. From this calculation, it was found that the CMCase exhibited a molecular mass of 33.2 kDa, which is indicative of a monomeric protein. The CMCase enzyme from *A. niger* VTCC F021 had a molecular mass of 31 kDa (Pham *et al.* 2012), while the endoglucanase enzyme

that was purified from *Trichoderma viride* had a molecular mass of 26.4 kDa (Cai *et al.* 2012). Two different endoglucanases were purified from *Trichoderma* sp. with molecular masses of 34 and 58 kDa, respectively (El-Zawahry *et al.* 2010). To check the further purity of the CMCase enzyme, the enzyme solution was loaded on to a Hypersil ODS column of high performance liquid chromatography. Results (Fig. 2) revealed that the enzyme showed a single peak at a retention time of 0.772 min, confirming that the enzyme solution was pure.

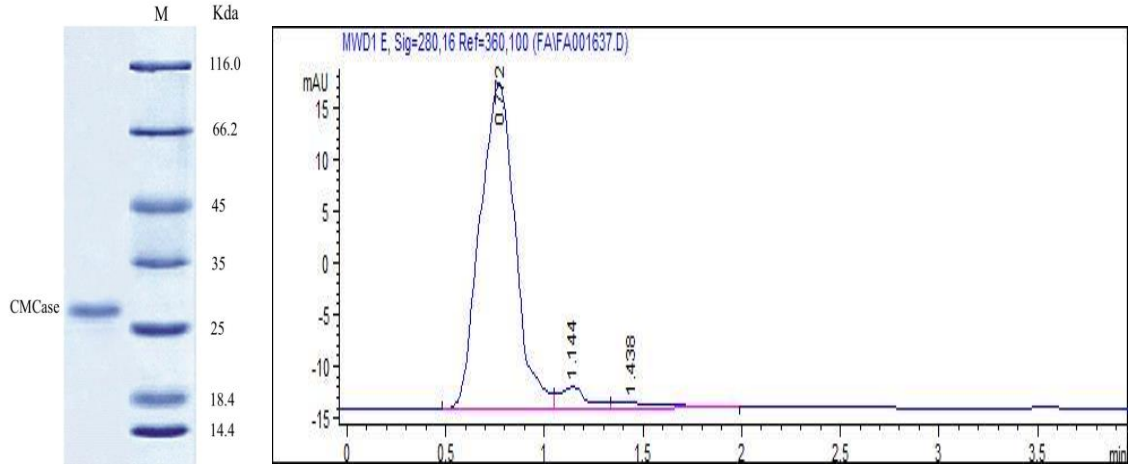


**Fig. 2.** Purity check of CMCase using (a) SDS-PAGE and (b) HPLC chromatogram

## FTIR Analysis of CMCase

The purified CMCase enzyme was characterized by FTIR spectroscopy. The IR spectrum of the purified enzyme indicated that there were some peaks in the 611.00 to 615.00 $cm^{-1}$ region, which belong to the secondary amide of the amide V and can be attributed to the out of plane NH bending (Elliot and Ambrose 1950). The peaks at 1240.00 $cm^{-1}$ and 1400.00 $cm^{-1}$ represent the secondary amide III bands and primary amide of the amide III bands, which are associated with the CN stretching vibration and NH bending vibration, respectively. CMCase enzyme had a strong absorption peak at 1655.46 $cm^{-1}$, which is a characteristic peak of the α-helix, caused by the symmetric stretching vibration of C=O stretching vibration and the NH bond (Dong *et al.* 1992; Susi and Byler 1986; Byler and Susi 1986). The bands between 1700 and 1600 $cm^{-1}$ are considered to be the most sensitive regions for protein secondary structural components (amide I band). These peaks are due to the C=O stretch vibrations of the peptide linkages (Kong and Yu 2007). The bands at 3100 $cm^{-1}$ and 3300 $cm^{-1}$ represent the amide B and amide A linkages, which are due to NH stretching (Elliot and Ambrose 1950; Krimm and Bandekar 1986; Banker 1992; Miyazawa *et al.* 1956).

## Ultraviolet Absorption Spectrum of CMCase

The purified CMCase enzyme solution was used for UV absorption spectrum by using buffer as control, the full band scan detection on a UV spectrophotometer. The results (Fig. 4) showed that the CMCase enzyme had a maximum absorption peak at 219.0 nm, indicating the presence of an aromatic side chain, especially the presence of tyrosine, tryptophan, phenylalanine, or their residues. The purified cellulase enzyme from *Aspergillus oryzae* ITTC-4857.01 exhibited maximum absorption peaks at 270 nm

(Begum and Absar 2009) and 290 nm (Begum 2005), indicating some structural resemblance to the enzyme.

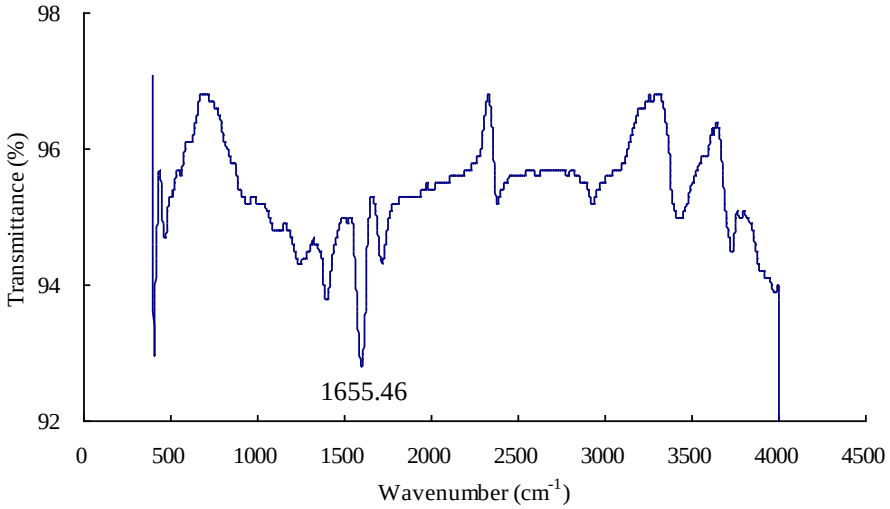


**Fig. 3.** FTIR spectrum of purified CMCase enzyme from *Penicillium simplicissimum*

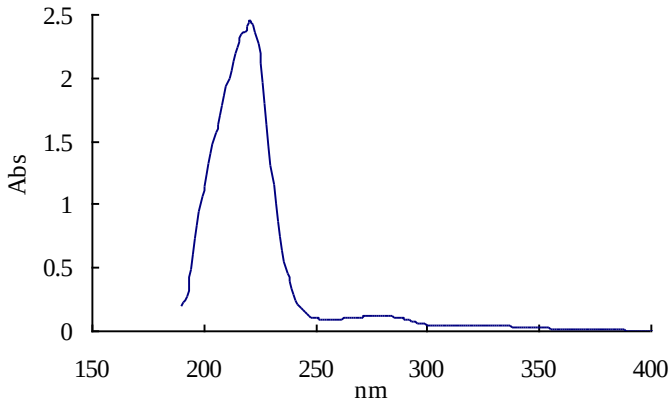


**Fig. 4.** Ultraviolet absorption spectrum of CMCase produced from *Penicillium simplicissimum*

## Characterization of CMCase

*Effect of temperature on activity and stability*

The activities of the CMCase were assayed at various temperatures (30 °C, 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C) to find the optimum temperature. Results (Fig. 5) indicated that enzyme activity increased with increases in temperature until peak activity was observed at 60 °C. After that, as the temperature was increased from 60 °C to 80 °C, a sharp decline in enzyme activity was observed. Raising the temperature to 80 °C caused denaturation of the enzyme. The thermostability profile of the enzyme showed that the endoglucanase enzyme was stable within the range 30 to 50 °C for 4 h. Thermostability at 50 °C for 4 h is beneficial for enzymatic hydrolysis of lignocellulosic biomasses. Most endoglucanases are optimally active in the temperature range of 50 to 70 °C, as shown in Table 2. For example, the endoglucanase enzyme from *A. niger Z10* was optimally active at 40 °C and retained 41.2% of the original activity at 90 °C of heat treatment for 15 min (Coral *et al.* 2002).

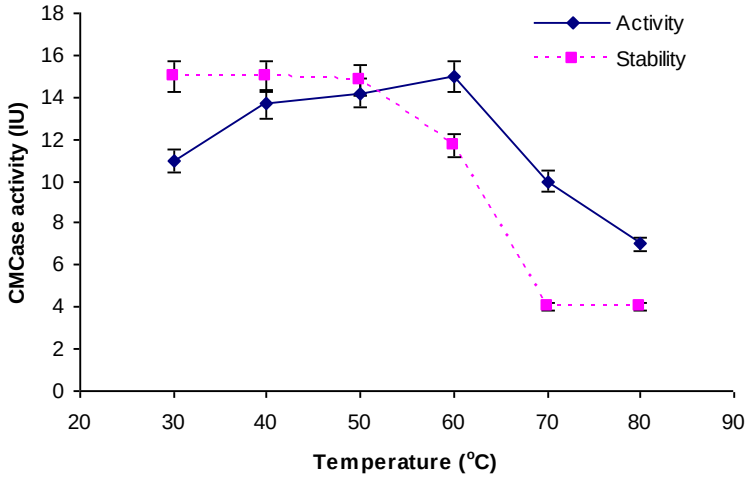


**Fig. 5.** Effect of temperature on CMCase activity and stability

*Effect of pH on activity and stability*

The activity of the enzyme was assayed using citrate buffer at various pH values (2.8, 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, and 6.8) at 50 °C for 30 min. The results (Fig. 6) indicated that the optimum pH of CMCase was 3.2. Any further increase in pH resulted in a decline in enzyme activity. To check the pH stability of the enzyme, the enzyme solution was pre-incubated at various pH for 12 h at ambient temperature (*i.e.*, 30 °C). The enzyme was stable in the pH range of 3.2 to 5.6. These findings were in good agreement with Karboune *et al.* (2008), who also reported pH stability in the range of 2.5-7.5 for endoglucanase from P. *funiculosum*. Table 2 compares the optimum pH of CMCase from various microorganisms. The endoglucanase enzyme from *A. glaucus* was active at pH 4 with a broad pH stability range from 3.5 to 7.5 for 4 h at 4 °C (Tao *et al.* 2010). Karboune *et al.* also reported that endoglucanase from P. *funiculosum* had a broad range of pH stability. The purified endoglucanases were stable in a broad pH range of 2-10 (Gao *et al.* 2008; Nazir *et al.* 2009; Akiba *et al.* 1995).

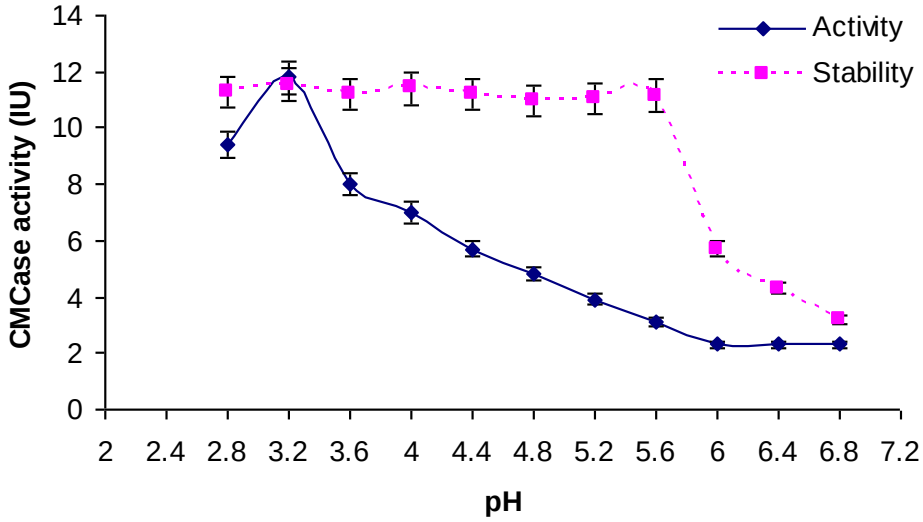


**Fig. 6.** Effect of pH on CMCase activity and stability

**Table 2.** Comparison of Optimum pH and Temperature of CMCase from Various Fungal Species

| Fungus | Opt. pH | Opt. temp. (°C) | Reference |
|---|---|---|---|
| *Penicillium simplicissimum H-11* | 3.2 | 60 | This study |
| *Penicillium funiculosum* | 4.0 | 65 | Karboune *et al.* (2008) |
| *Aspergillus niger VTCC-F021* | 5 | 55 | Pham *et al.* (2012) |
| *Chaetomium cellulolyticum NRRL 18756* | 5.5 | 50 | Fawzi and Hamdy (2011) |
| *Trichoderma sp.* | 5.0 | 50 | El-Zawahry *et al.* (2010) |
| *Trichoderma viride* | 6.5 | 55 | Iqbal *et al.* (2011) |
| *Aspergillus niger ANL301* | 5.5 | 50 | Chinedu *et al.* (2011) |
| *Trichoderma sp. IS-05* | 3.0 | 60 | Andrade *et al.* (2011) |
| *Daldinia eschscholzii (Ehrenb.:Fr.) Rehm* | 6.0 | 70 | Karnchanatat *et al.* (2008) |
| *Aspergillus glaucus XC9* | 4.0 | 50 | Tao *et al.* (2011) |
| *Aspergillus Oryzae VTCC-F045* | 5.5 | 55 | Nguyen and Quyen (2010) |

*Effect of metal ions*

To obtain the effect of metal ions (10 mmol/L), the enzyme was treated with different metals for 30 min at 50 °C. After that, the enzyme activity was determined using a standard procedure. The results showed that $Mg^{2+}$ (150.16%) and $Sn^{2+}$ (110.44%) were strong activators, while $Cu^{2+}$ (3.89%) was a strong inhibitor of the CMCase enzyme (Table 3). The stimulation and inhibition of the enzyme activity depends on the active site of the enzyme. These findings were mostly in accordance with other studies. CMCases of other fungal species are strongly activated by $Co^{2+}$, $Mn^{2+}$ (Iqbal *et al.* 2011), Cu,$^{2+}$ $Fe^{2+}$ (Pham *et al.* 2012), $Fe^{2+}$ (Chen *et al.* 2001), $Co^{2+}$, and $Zn^{2+}$ (Elshafei *et al.* 2009) and mostly inhibited by $Hg^{2+}$ (Iqbal *et al.* 2011; Elshafei *et al.* 2009; Gao *et al.* 2008; Akiba *et al.* 1995).

**Table 3.** Effect of Metal Ions on CMCase Activity

| Metal ions | CMCase relative activity (%) |
|---|---|
| Control | 100.00 |
| $Mn^{2+}$ | 42.64 |
| $Sn^{2+}$ | 110.44 |
| $Zn^{2+}$ | 89.30 |
| $Cu^{2+}$ | 3.89 |
| $Ca^{2+}$ | 93.90 |
| $Mg^{2+}$ | 150.16 |
| $Co^{2+}$ | 56.98 |
| $Li^{+}$ | 63.66 |
| $Fe^{2+}$ | 66.82 |
| $Fe^{3+}$ | 96.10 |

*Substrate specificity of the enzyme*

The purified CMCase enzyme was used to check the substrate specificity by reacting with various substrates such as filter paper, microcrystalline cellulose (1%), xylan (1%), CMC-Na (1%), chitin (1%), and salicin (0.5%). From the results (Table 4), it was observed that the enzyme CMCase could effectively hydrolyze microcrystalline cellulose (17.39 ± 0.29), CMC-Na (15.39 ± 0.25), and filter paper (11.74 ± 0.50 IU). These results indicated that this enzyme had properties of both endo- and exo-glucanase,

which is an interesting feature of this study. This bifunctional nature of cellulase enzyme has been reported from few fungi (Oliveira *et al.* 2013; Boisset *et al.* 2000; Liu *et al.* 2001), but mostly from bacterial species (Bao *et al.* 2011; Ko *et al.* 2011; Sakon *et al.* 1997). This enzyme degraded xylan and chitin to some extent, but it had no activity against salicin. In most studies, endoglucanase had strong activity against CMC-Na (Pham *et al.* 2012; Iqbal *et al.* 2011; Chinedu *et al.* 2011; Karnchanatat *et al.* 2008).

**Table 4.** Substrate Specificity of CMCase Produced from *Penicillium simplicissimum H-11* in Submerged Fermentation

| Substrates | Enzyme activity (IU) |
|---|---|
| Filter paper | 11.74 ± 0.50 |
| Microcrystalline cellulose (1%) | 17.39 ± 0.29 |
| Xylan (1%) | 3.50 ± 0.19 |
| CMC-Na (1%) | 15.39 ± 0.25 |
| Chitin (1%) | 3.52 ± 0.03 |
| Salicin (0.5%) | 0.00 ± 0.00 |

*Enzyme kinetics*

The kinetic parameters $K_m$ and $V_{max}$ of the CMCase enzyme were estimated by a Lineweaver-Burk plot using various concentrations of salicin as a substrate. The main purpose of estimating kinetics is to obtain the catalytic efficiency of proteins. Results (Fig. 7) revealed that the $K_m$ and the $V_{max}$ of CMCase were 14.881 mg/mL and 0.364 mg/mL/min, respectively.

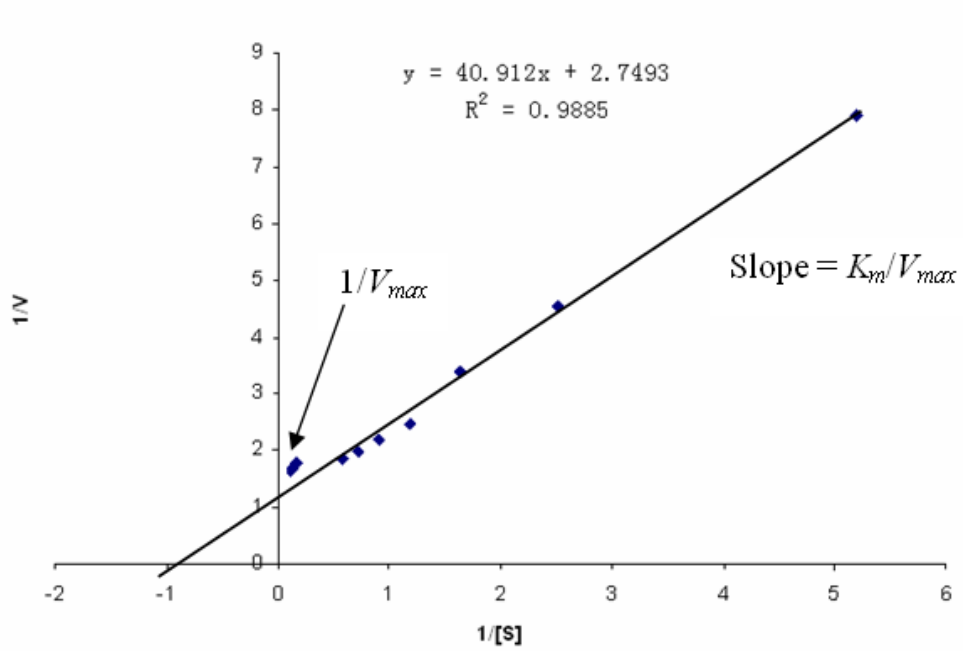


**Fig. 7.** Lineweaver-Burk plot for determination of kinetic constants against CMC-Na.

$K_m$ is the dissociation constant, which represents the affinity of substrate in enzyme substrate (ES) complex. This $K_m$ (14.881 mg/mL) value is lower than that of *A. niger* (52-80 mg/mL) (Hurst *et al.* 1977), and higher than that of *A. niger* VTCC F021 (8.5815 mg CMC/mL) (Pham *et al.* 2012) and *A. awamori*VTCC-F099 (5.83 mg/mL)

(Nguyen and Quyen 2010). However, the $V_{max}$ (0.364mg/mL) value was lower than earlier work (Pham *et al.* 2012; Nguyen and Quyen 2010; Nazir *et al.* 2009; Elshafei *et al.* 2009). *Trichoderma viride* endoglucanases have $K_m$ and $V_{max}$ values of 68 µM and 148 U/mL, respectively, against CMC-Na (Iqbal *et al.* 2011).

## CONCLUSIONS

1. The strain *Penicillium simplicissimum* H-11 has the ability to produce cellulase enzyme in submerged fermentation using wheat straw as a substrate.
2. The protein produced by this fungus exhibits a secondary structure, particularly the α-helix structure.
3. Due to the broad pH and temperature stability and its bifunctional (endoglucanase and exoglucanase) nature, this enzyme could be used in enzymatic hydrolysis, especially in saccharification of lignocellulosic biomasses for ethanol production.

## ACKNOWLEDGMENTS

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