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Purification and characterization of cellulase from *Aspergillus unguis* isolated from decaying coconut wood

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ABSTRACT

The insufficiency of available natural fossil fuels for increasing human population, air pollution due to partial combustion of fossil fuel and the release of greenhouse gasses have led great attention on the usage of cellulase like enzymes to hydrolyze lignocellulosic substances to produce bioethanol. Thermostability and kinetic properties of cellulases need to be studied before deciding the eligibility of the enzymes for their potential applications. This study was aimed to purify the crude cellulase from Aspergillus unguis and to characterize the purified cellulase. When the crude enzyme from Aspergillus unguis isolated from decaying coconut wood, *was subjected to fractional precipitation and dialysis by the addition of 80% saturated $(NH_4)_2SO_4$, the recovery of cellulase was 83.9 % showing specific activity of 16386.43Umg^{-1} protein. The dialyzed enzyme was added to a column packed with DEAE-Sepharose equilibrated with 0.01M sodium phosphate buffer (pH 7.0) and unbound proteins were washed with the same buffer. The specific activity of cellulase was increased from 3228 to 37071 Umg^{-1} protein, which was 11.5-fold higher than that of the crude cellulase with 67.6 % yield. The molecular weight of the purified cellulase was determined as 50 KDa using Poly Acrylamide Gel Electrophoresis (SDS-PAGE). When the activity of purified cellulase was measured at different temperatures ranging from 40^{o}C to 90^{o}C at neutral pH, the optimum temperature for the activity of the purified cellulase enzyme was 70^{o}C. The pH was optimized as 5.0 for the cellulase at 70^{o}C. Michaelis constant for the purified cellulase to soluble cellulose by Lineweaver-Burk Plot was 4.45 ×10^{-2} moldm^{-3} and V_{max} was 28.5714 mgml^{-2}mins^{-1} with 10 gL^{-1} of cellulose substrate, at pH 5.0 and 70^{o}C. The purified cellulase was stable for at least 90 minutes at pH 5.0 and 70^{o}C and the half-life obtained for this enzyme was significantly higher at 70^{o}C than any other temperatures. Therefore, the crude cellulase from Aspergillus unguis can be purified by ammonium sulphate precipitation and DEAE-sepharose ion exchange chromatography. The thermostable acidic cellulase from Aspergillus unguis could be a potential candidate for diverse industrial applications.*

Key words: *Aspergillus unguis, cellulase, kinetic properties, michaelis constant, purification.*

INTRODUCTION

Bioconversion of lignocellulosic materials into efficient bioactive products is the principle step to establish a vigorous biological economy. This process needs an initial pretreatment step followed by an enzyme hydrolysis to produce fermentable sugars. Ionic liquid pretreatment is a highly advanced method to induce the biomass saccharification by cellulase enzyme. Major disadvantage of this method is that the remaining ionic liquid in the hydrolysis buffer will hinder the cellulase activity. Efficient enzyme hydrolysis will overcome the recalcitrance of lignocellulosic biomass to produce fermentable sugars. The industrialization process of the cellulase production through pretreatment and hydrolysis, involves in major cost reduction and utilization of maximum resources (Juturu and Wu, 2014). Currently cellulases have created considerable attention and the demand for the production has been showing an increasing trend because of their usage in diverse industries such as

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food, textiles, pulp and paper and agriculture and land use (Costa *et al*., 2008). Increasing rate of greenhouse gas emission due to substantial incidences of partial burning of fossil fuel have resulted in the usage of cellulases for the hydrolysis of the lignocellulosic waste materials for producing bioethanol (Zaldivar *et al*., 2001). Cellulases consist of three different enzymes such as endo-1,4- β-glucanase (Endogluconase), exo-1,4-β-glucanase (Exoglucanases) and β-glucosidase. These enzyme combinations hydrolyze the cellulose substrate into soluble sugars and glucose (Lynd *et al*., 2002). Crystalline macroscopic structure of cellulose will be broken down by endogluconases and results in short linear chains of glucose molecules. Exo-glucanases will react on the broken pieces of short chains and this would yield cellobiose and glucose. Saccharification by β-glucosidases will break cellobiose and small cello-oligosaccharides into basic glucose molecules (Harrison *et al*., 1998). Cellulase production could be induced by microorganisms when they grow on appropriate cellulosic materials under condusive environmental conditions. Different types of cellulases are produced by major microorganisms classified under fungi, bacteria and actinomycetes (Gaur & Tiwari, 2015).

Multicellular fungal decomposers have been substantially used for breaking down cellulosic materials all around the globe (Moore Landecker, 1996). These fungi can grow well on diverse wood types and the growth of the fungi has been extremely high during the natural degradation of wood components. Members of the ascomycota, basidiomycota, and deuteromycota are considered as efficient fungal decomposers. The cellulolytic fungul species include *Aspergillus, Fusarium, Penicillium, Trichoderma, Alternaria* etc. (Mehrotra and Aneja, 1990). The fungal cellulases produced by *Aspergillus, Trichoderma,Penicillium* and *Sclerotium* are of higher importance for the industrial applications because of their capacity to produce comparatively substantial quantities of cellulases and their extracellular and adaptive nature in diverse environmental conditions (Mathew *et al*., 2008). *Trichoderma reesei* has been reported as one of the best native cellulose hydrolyzers (Singhania *et al*., 2006).

Thermophilic fungi are highly preferred for cellulase extraction because of the swelling up of cellulose fibers at higher temperatures would facilitate the easy extraction of the hydrolytic enzymes (Li *et al*., 2011). Thermophilic fungus *Talaromyces emersonii* produced active cellulase at 70°C (Murray *et al*., 2004). Two strains of *Penicillium* that were isolated from soil of subtropical region produced functional extracellular cellulase enzymes (Picart *et al*., 2007). Thermostable cellulases are produced in high phase by organisms such as *Chaetomium thermophilum, Sporotrichum thermophile, Talaromyces emersonii* and *Thermoascus aurantiacus* (Li *et al*., 2011). Extracellular thermostable cellulases are potentially useful in the biotechnological and industrial applications since they play vital role in the bioconversion of cellulosic biomass to different value-added biomaterials. Therefore, the objective of this study was to purify the crude cellulase from *Aspergillus unguis* and to determine the kinetic properties and stability of the purified cellulase.

MATERIALS AND METHODS

Microorganism

Among the different types of fungal colonies isolated from different lignocellulosic materials, colonies that produced higher quantity of cellulase enzyme were cultured initially. The best cellulase producer, isolated from decaying coconut wood was identified as *Aspergillus unguis* and used in this study.

Chemicals and Media

All the chemicals used here were from standard sources. The activation medium contained cellulose 0.4g and nutrient broth 0.8g in 100ml of distilled water. Fermentation medium contained (gL^{-1}) cellulose 2.0g; carboxymethyl cellulose 0.3g; ammonium sulphate 0.3g and incubated at room temperature (27^{o}C), pH 7.0 and 100 rpm for 6 days.

Purification of cellulase

To the fermentation medium, 24 h old cultures of *Aspergillus* inoculum (size $6x10^6$ conidia) was added and incubated at 30^{o}C in an orbital shaker (at 100rpm). Samples were removed from the shaker after 6 days and centrifuged and the supernatant was used as the cellulase source and subjected for purification.

The crude culture filtrate was cool centrifuged at 10,000 rpm to remove the cells and the residual medium. Supernatant was precipitated overnight with $(NH_4)_2SO_4$ (80% saturation) and the pellet was recovered by centrifugation at 12000 rpm for 10 min. The pellet was re-suspended in a small amount 100 mM phosphate buffer (pH 7.0) and dialyzed overnight with 80% saturated $(NH_4)_2SO_4$ against the same buffer. The corresponding precipitates were recovered, dissolved individually in buffer and assayed for both total protein content and cellulase activity. The dialyzed sample was loaded to a DEAE-Sepharose (Sigma-Aldrich, USA) column equilibrated with sodium phosphate buffer (100 mM, pH 7.0).

The enzyme fraction was allowed to bind with matrix for 2 h at 4°C. The unbound fraction was collected and analyzed for the cellulase enzyme activity and for protein content. The bound fractions were eluted with a NaCl gradient (0.1-0.5 M, 10 ml each) in the same buffer at a regular flow rate of 1.0 ml min^{-1}.

Polyacrylamide gel electrophoresis

The active fraction, showing higher specific activity, collected from the Sepharose column filtration was electrophorized by Sodium Dodecyl Sulphate-Poly Acrylamide gel electrophoresis with 12.5% polyacrylamide gel (Kapilan and Arasaratnam, 2014). The molecular weight of the cellulase was estimated by SDS-PAGE against the molecular mass markers that ran with the samples.

Kinetic studies

Activity of cellulase with time

Soluble cellulose ($1gml^{-1}$ 0.25ml, pH 5.0) was mixed with purified cellulase (0.25mL) at 70^{o}C in triplicates and the amount of glucose produced was monitored. Activity of the purified cellulase was determined as a function of time with soluble cellulose.

Effect of temperature

The effect of temperature on cellulase activity was determined by incubating the appropriately diluted (10 fold dilution) cellulase enzyme (pH 5.0) for 5 minutes with 0.25mL of soluble cellulose at pH 7.0, 10 ml (1g /100ml) and at different temperatures such as 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C in triplicates. The enzyme and substrate samples at pH 7.0 were preincubated at the respective temperatures considered for the studies for 5 minutes and then mixed to determine the activities.

Effect of pH

The effect of pH on cellulase activity was measured by preparing 1g/100ml soluble cellulose in phosphate buffers at different pH values ranging from 2.0 to 8.0 in triplicates and incubated at 70^{o}C. The enzyme and substrate samples at respective pH values were preincubated at 70^{o}C for 5 minutes.

Effect of substrate concentration

Different concentrations of cellulose solutions were prepared (0.25 to 40gL-1) in citrate -phosphate buffer at optimum pH in triplicates and they were allowed to react with the diluted enzyme at optimum temperature for optimized time. The enzyme activity was measured. The Michelis constant and V_{max} for the cellulase to cellulose were calculated by plotting Lineweaver-Burk graph.

Effect of temperature on the stability of cellulase

Stability of the purified cellulase preparations was determined, at the optimum pH (5.0) by incubating the enzyme at different temperatures such as 60^{o}C, 70^{o}C and 80^{o}C. The enzyme activities were assayed under optimized conditions.

Analytical methods

Cellulase activities were assayed according to the modified method described by Ghose (1987). The glucose produced was measured by Dinitro salicylic acid (DNSA) method. One unit of cellulase activity is defined as the amount of enzyme that produces one µmol of reducing sugar in one minute at pH 5.0 and 50^{o}C with 10 ml (1g/100ml) cellulose.

The values of enzymatic activity were expressed as U/ml. Reducing sugar was measured by DNS method (Miller, 1959).

Protein concentration was determined by Lowry's method (Lowry *et al*., 1951). Each experiment was performed in triplicate and the mean and standard deviation for each experiment were presented.

Statistical analysis

All the experiments were made in triplicates and the mean values were used to plot the graphical representation. Statistical analyses were performed using R 2.15.3 (R Development Core Team, 2010). The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine the significant differences at $p \leq 0.05$.

RESULTS AND DISCUSSION

Purification of cellulase

When the crude enzyme was precipitated by fractional precipitation method by the addition of solid $(NH_4)_2SO_4$ from 10 to 90% saturation, highest specific activity (36.5 Umg^{-1}protein) was precipitated at 80% solid $(NH_4)_2SO_4$ saturation. The protein precipitated with 80% saturation of $(NH_4)_2SO_4$ was collected and dissolved in sodium phosphate buffer (pH 7.0) and dialyzed against the same buffer. This fraction had 16386.43 U mg^{-1} of specific activity with the recovery of 83.8% and the purification fold for this step was 5.08 (Table 1). When the active fraction of ammonium sulphate was subjected for further purification by using DEAE-Sepharose ion exchange chromatography and the dialyzed enzyme was added to a column packed with DEAE-Sepharose equilibrated with 0.01M Sodium phosphate buffer (pH 7.0) the specific activity of cellulase was increased from 3228 to 16386 Umg^{-1} protein, which was 11.5 fold higher than that of the crude cellulase with 67.6% yield.

One mL of sample was loaded into the DEAE-Sepharose column pre-equilibrated with sodium phosphate buffer (100 mM at pH 7.0) and allowed to travel through the column. When the un-bound fraction was collected and analyzed for cellulase enzyme activity and protein content, the cellulase activity was absent in the un-bound fraction but protein was estimated as 1.78 mg ml^{-1}. Absence of enzyme activity in the un-bound fraction lead to a finding that the total cellulase present was bound to the matrix. When the bound cellulase enzyme was eluted by sodium phosphate buffer (100 mM, pH 7.0) having NaCl with

increasing concentration gradient (0.1 M to 1.0 M, 10 ml of NaCl solution of each concentration), the cellulase enzyme activity was detected in the small fraction released by the addition of 0.5 M NaCl. One prominent peak was observed at the 20^{th} fraction (Figure 1).

Separation of purified cellulase on SDS-PAGE and determination of the molecular weight

When the pooled purified cellulase mix was run in the gel electrophoresis and stained with ethidium bromide, the sample gave a very clear single band (Figure 2). The molecular weight of the purified cellulase was determined by the method developed by Weber and Osborn (1969). The distance traveled by molecular markers and purified cellulase were measured. The linear relationship existed between the logarithm of molecular weight of molecular markers and distance migrated by molecular markers. The molecular weight of the purified cellulase was estimated as 50 kDa.

Table 1: Purification profile of cellulase enzyme from *Aspergillus unguis* on ammonium sulphate and Sepharose anion-exchange chromatography.

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	381788	118.25	3228.65	100	1.00
Ammonium sulphate addition	320191	19.54	16386.43	83.87	5.08
Sepharose anion-exchange chromatography	258012	6.96	37070.75	67.58	11.48

Note: Specific activity was calculated dividing the total activity by total protein at a particular purification step. Purification fold was calculated dividing the specific activity of a particular purification step, by specific activity of crude enzyme sample. Recovery (%) was calculated dividing the total enzyme activity of a particular purification step by total enzyme activity of crude enzyme sample and multiplied by 100.

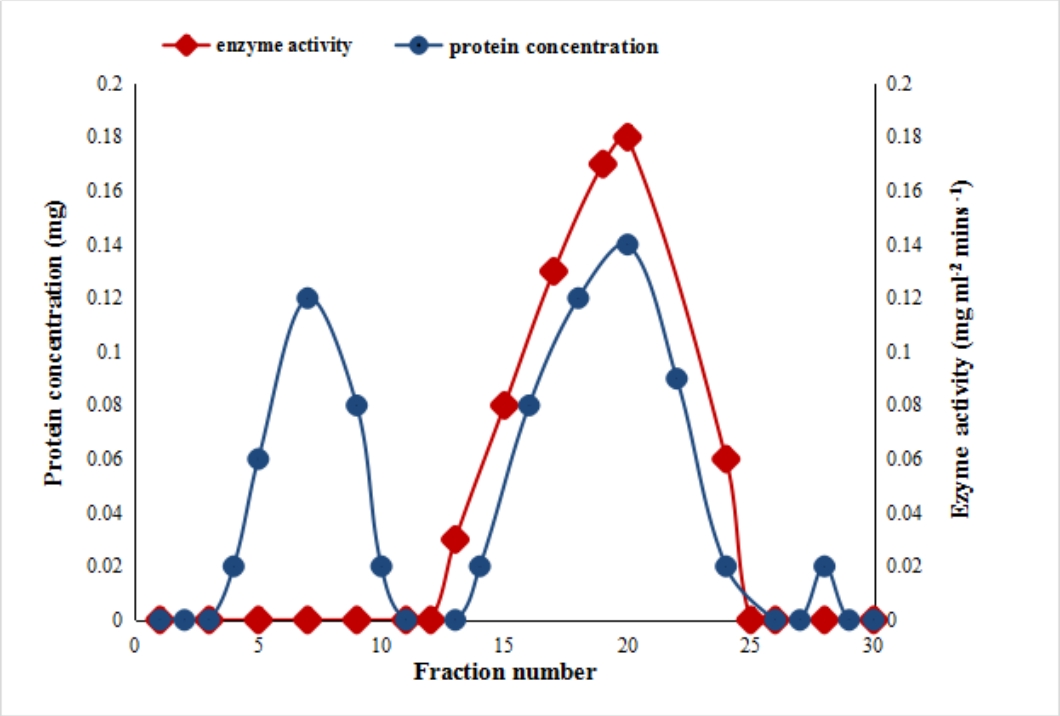

Figure 1: Purification profile of the cellulase of *Aspergillus unguis* using ammonium sulphate and Sepharose anion-exchange chromatography C- 25 fast flow column (1.5×25 cm). (●), cellulase activity (UmL^{-1}) eluted with 0.8M NaCl containing 0.01M phosphate buffer (pH 7.0); (▲), Protein (mg) eluted with 0.8M NaCl containing 0.01M phosphate buffer (pH 7.0) were used at flow rate of $1mLmin^{-1}$ to wash the unbound protein.

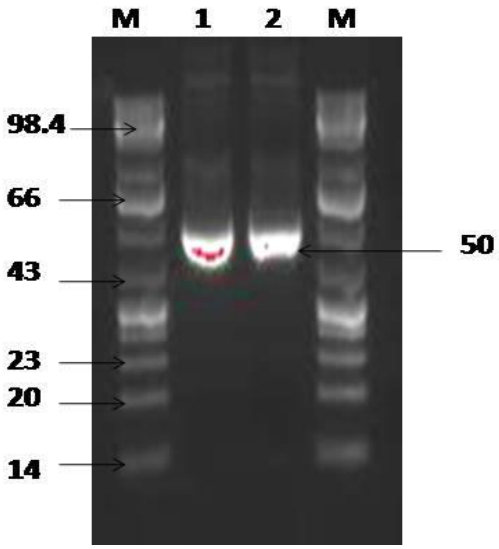

Figure 2: SDS-PAGE of different fractions of cellulase of *Aspergillus unguis* obtained in the purification. Lane 1: Marker; lane 1: Concentrated cellulose enzyme after ammonium sulphate precipitation lane 2: Purified cellulase enzyme after gel-filtration chromatography. Molecular weights are mentioned in kDa.

Activity of cellulase with time

Purified cellulase of the triplicate samples showed zero order kinetics for 5 minutes. Therefore, the incubation time for the kinetic studies was fixed as 05 minutes for further experiments.

Effect of temperature

When the cellulolytic activity was assayed at different temperatures ranging from 40°C– 90°C at pH 7.0, significantly higher activity was obtained at 70°C (17.1052mg $ml^{-2}mins^{-1}$, (Figure 3)) than the other temperatures tested (p=> 0.05).

Effect of pH

When the initial pH of the media was changed, significantly higher cellulase activity was obtained at pH 5.0 than the other pH values tested (4.0 to 8.0, Figure 4). Cellulase activity was observed in all media maintained at different pH values. Since significantly higher activity was obtained at pH 5.0, this pH value was chosen for further studies.

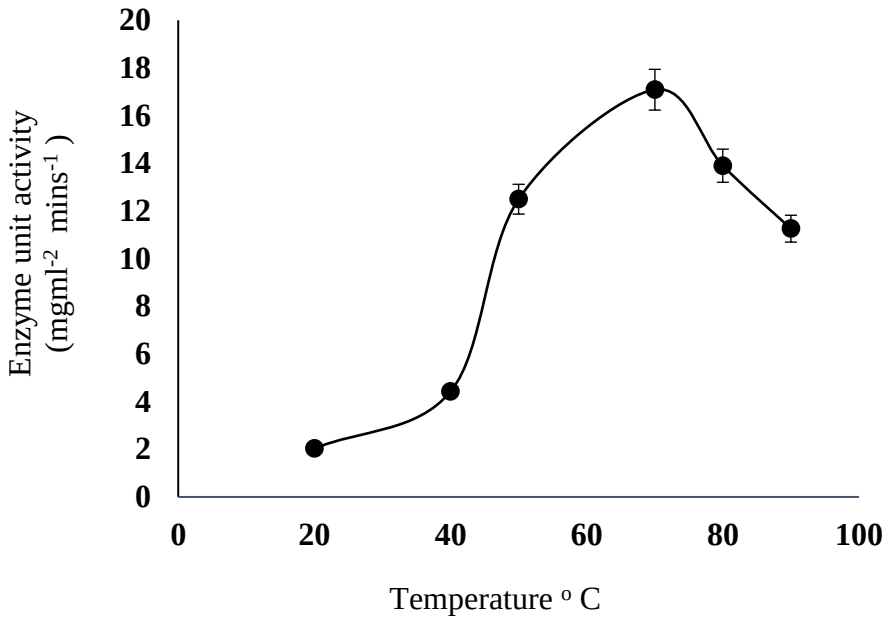

Figure 3: Effect of temperature in purified cellulase enzyme activity of *Aspergillus unguis* obtained from decaying coconut wood.

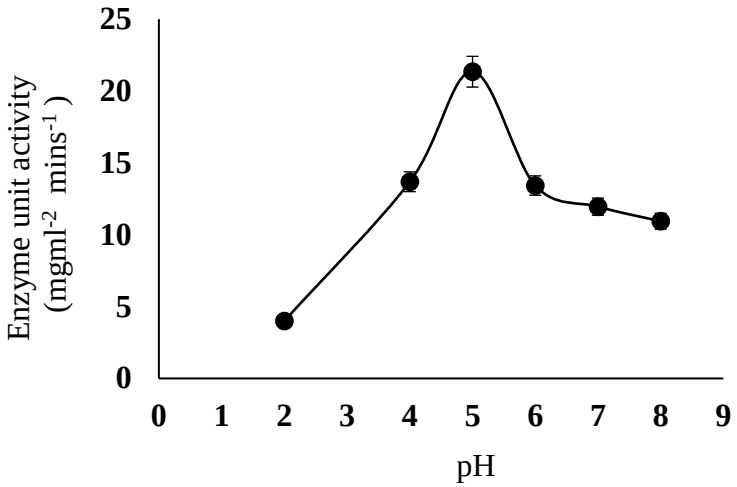

Figure 4: Effect of pH of the media on the purified cellulase of *Aspergillus unguis* obtained from decaying coconut wood.

Effect of substrate concentration

When the cellulose concentration was increased from 0.25 to 40gL^{-1} at pH 5.0 and 70^{o}C, the cellulase activities obtained beyond the concentration of 10gL^{-1} were significantly higher than all the other lower concentrations tested (Figure 5). Therefore 10gL^{-1} was fixed as the cellulose concentration for all the future experiments. Michaelis constant for the purified cellulase enzyme to soluble cellulose by Lineweaver-Burk Plot was 4.45×10^{-2}moldm^{-3} and Vmax was 28.5714mg ml^{-2}mins^{-1} at PH 5.0 and at 70^{o}C (Figure 6). Therefore 10ml (1g/100ml) substrate was used for the purified cellulase enzyme assay.

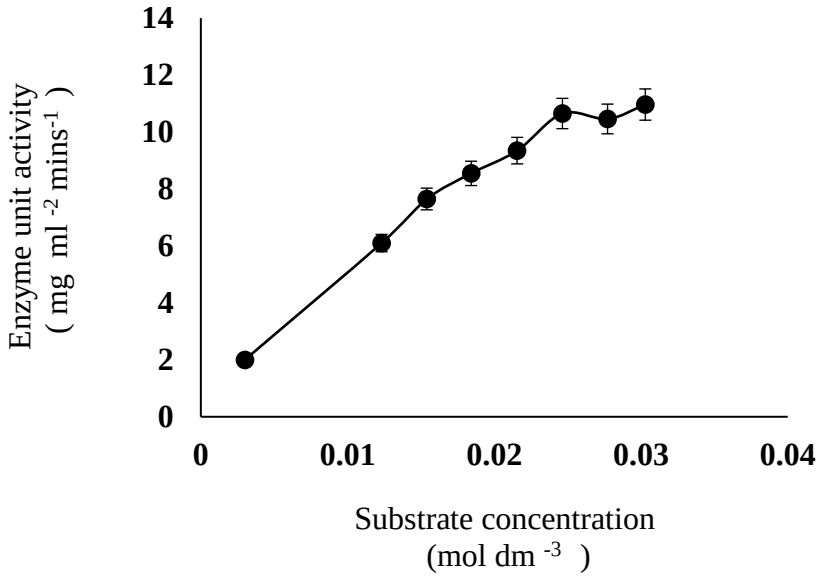

Figure 5: Effect of different substrate concentration on the purified cellulase enzyme of *Aspergillus unguis* isolated from coconut wood.

Effect of temperature on the stability of cellulase

When the purified cellulase was pre-incubated at different temperatures (60, 70 and 80^{o}C) for a period of 100 minutes, the activity significantly decreased with time. The enzyme was stable for at least forty minutes at all the temperatures tested. When the purified enzyme was pre-incubated at 70^{o}C for 60 minutes, more than 70 % of the mean original activity was retained. When the purified enzyme was pre-incubated at 70^{o}C, 95% of the mean original activity was retained at 20 min, while at 90 min, 18 % of the mean original activity was retained. After 80 minutes of incubation, the half-life of the purified cellulase was 32mins and 19 sec when cellulase was incubated at 70°C (Figure 7). When the purified cellulase was pre-incubated at 70^{o}C for one hour, 25.5 % of the mean original activity was retained and this activity was significantly higher than that of 60^{o}C and 80^{o}C.

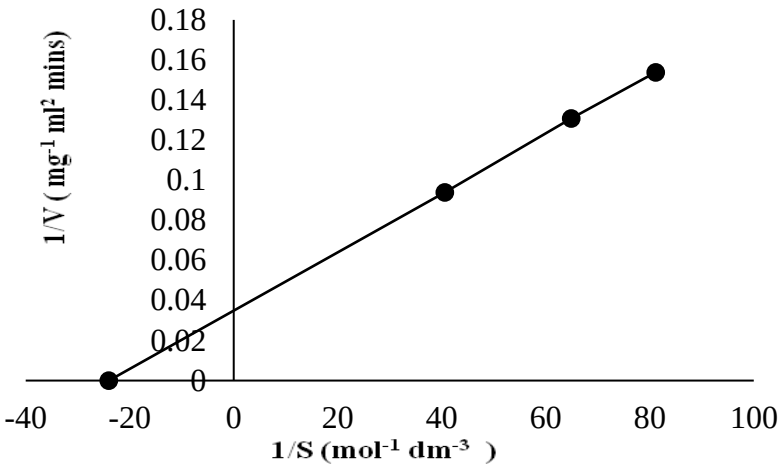

Figure 6: Lineweaver-Burk plot of the purified cellulase from *Aspergillus unguis* at pH 5.0 and 70^{o}C using different concentrations of cellulose substrate

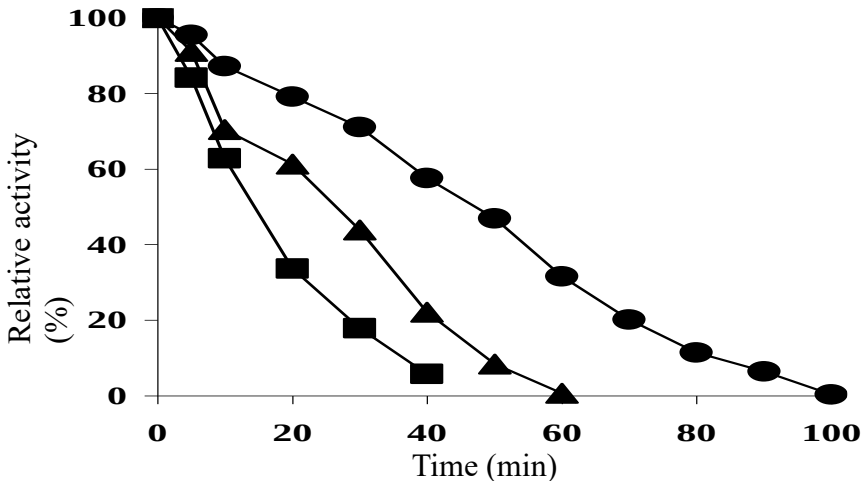

Figure 7: Stability of purified cellulase of *Aspergillus unguis* at different temperatures of (▲), 60; (●), 70 and (■), 80 at pH 50^{o}C with 10gL^{-1} cellulose (0.01M phosphate buffer) at pH 5.0.

Fungal species that can produce diverse lignocellulolytic enzymes play vital role in the bioremediation, biogeochemical cycles and nature's balance. This study was targeted on the purification and characterization of extracellular cellulase enzyme produced by *Aspergillus unguis* after being grown in fermentation medium for 6 days at 100 rpm. The crude cellulase enzyme from *Aspergillus unguis* was purified from the spent medium by 80% saturated ammonium sulphate precipitation and by ion exchange chromatography using DEAE-sepharose fast flow and the molecular weight was determined as 50 KDa using SDS-PAGE. The cellulase enzyme from *Bacillus vallismortis* RG-07 was purified by $(NH_4)_2SO_4$ precipitation, ion exchange and gel filtration chromatography and the final recovery was 28.8%. Using the SDS-PAGE and activity gel analysis the molecular weight of purified cellulase from thermostable alkalophilic cellulase *Bacillus vallismortis* RG-07 was determined as 80 kDa (Gaur & Tiwari, 2015).

Different cellulases were separated and purified from fungal species *Aspergillus nidulans* based on the affinity of the enzyme towards substrate and the molecular weights were recorded for various enzyme forms as Exo-I, 29000; Exo-II, 72500; Exo-III, 138000; Endo-I, 25000; Endo-II, 32500; β-Gluco-I, 14000 and β-Gluco-II, 26000 (Bagga *et al*., 1990). When purified cellulase from *Aspergillus unguis* was subjected for the gel electrophoresis run, there was a very clear single band. This single band proved that *Aspergillus unguis* produces only one type of cellulase enzyme. The molecular weight of the purified cellulase was 50 kDa. The molecular weight of this purified cellulase is closely resembled the molecular weight (55000Da) of the Endoglucanase (Arja, 2004). The molecular weight of the active cellulases produced by diverse fungal species varies between 12 kDa and 126 kDa (Bai *et al*., 2013). The molecular weight (50 kDa) of the purified cellulase produced by *Aspergillus unguis* falls in this range. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is the most commonly used method for determining the exact molecular weight of the diverse microbial enzymes (Joo *et al*., 2010, Lee *et al*., 2010 & Ramani *et al*., 2012). Fungal cellulases are either monomeric (Naika *et al*., 2007) or dimeric (Chaabouni *et al*., 2005) in nature. Cellulase produced by *Trichoderma viride* was purified to homogeneity using DEAE-Sepharose column and the molecular weight was determined as 87 kDa by SDS-PAGE (Yasmin *et al*., 2013). Molecular weight of the monomeric cellulase produced by *Penicillium pinophilum* MS 20 was 42kDa and a single band was visible on SDS- PAGE gel (Pol *et al*., 2012). Strain *Aspergillus awamori* VTCC - F099 produced monomeric thermostable cellulase which appeared as a single band on SDS-PAGE gel with the molecular weight of 32 kDa (Van Tuan Nguyen *et al*., 2010). In all the above mentioned research studies including ours, the purified cellulase formed a single band on the SDS-PAGE gel. This led to a conclusion that most of the fungal cellulases are active as monomers or homodimers and they migrate through the SDS-PAGE gel based on their molecular weight (Coral *et al*., 2002).

Purified cellulase enzyme obtained from *Aspergillus unguis* showed significantly higher activity at 70^{o}C and at pH 5.0 than the other tested conditions. Characterization revealed that the purified cellulose enzyme produced by *Aspergillus unguis* was optimally active at acidic pH and thermostable conditions. This purified cellulase showed optimum activity at 70^{o}C, indicating it is more stable at 70^{o}C than any the other tested temperatures. Purified cellulase from *Aspergillus* sp was active and thermally stable at pH 7.0 and 70^{o}C (Anwaret *et al*., 2014). A filamentous fungus *Trichoderma harzianum*, produced cellulose degrading enzymes at 28^{o}C

(Bashir, 2009) and pH 5.5 (Sibtain *et al*., 2009). Purified cellulase from *Aspergillus niger* showed maximum stable activity at 45^{o}C (Hurst *et al*.,1997). The purified cellulase from *Trichoderma viride* showed its optimum activity at 50^{o}C (Abdul Sattar *et al*., 2014). Most cellulases from fungi are mesophilic, and the half-life of endoglucanase activity is typically less than 2 hours at 60^{o}C (Batt, 2000). *Aspergillus fumigatus* that was isolated from hyperthermal compost, showed significantly higher thermostability at 70^{o}C after 15 hours than the other temperatures (Liu *et al*., 2010). b-Glucosidase from *Penicillium citrinum* showed an optimum temperature of 70^{o}C, but its half life was 2 hours at 58^{o}C (Ng *et al*., 2010).

Based on the above literature and the results obtained in this study, it could be concluded that the purified cellulase from *Aspergillus unguis* is significantly functional under moderately acidic media conditions than the alkaline environments tested. This pure cellulase enzyme retained 25.5 % of its original activity at 70^{o}C for one hour and the capacity to retain the original activity slowly and steadily reduced with the time. The decrease in the activities of purified cellulase at the extreme acidic and alkaline pH values and higher temperatures might be due to the breakdown of the active site of the enzyme. Extreme alkaline pH might cause changes in the secondary and the tertiary structure of the cellulase structural proteins. The enzymes especially cellulases that are stable at acidic pH and high temperatures have great potential for the application in biotechnology (Mohanappriya and Kapilan, 2018) . These are the important properties that are considered to be highly wanted for the industrial saccharification of cellulose containing natural sources. The influence of pH and/or temperature on the enzymatic hydrolysis of cellulose within the selected range in this study is very small, therefore this cellulase enzyme could be a choice for industrial usage.

The Michaelis-Menten Constant (Km) is defined as the substrate concentration at half of the maximum velocity Vmax. Both kinetic parameters (Km = 4.45 $\times 10^{-2}$ moldm^{-3} and Vmax = 28.5714 mgml^{-2}mins^{-1}at pH 5.0 and 70^{o}C) of purified cellulase from *Aspergillus unguis* were determined by Lineweaver-Burk Plot for the cellulose substrate. Purified cellulases of different fungal and bacterial species show diversity in their Km and Vmax values. The Km and Vmax values of cellulase from *A. anitratus* and *Branhamella* sp. were 4.97 mM and 7.90 mg/mL respectively for carboxymethyl cellulose (Ekperigin, 2007) while the Km and Vmax values of cellulase from *Pseudomonas fluorescens* were 3.6 mg/mL and 1.1 mM (Bakare *et al*., 2005). The difference in Km and Vmax values of the purified cellulase from *Aspergillus unguis* and other fungal species may be due to the difference in the genetic constitution of the fungal species concerned and the degree of adaptation potential to the culturing environment (Iqbal *et al*., 2011).

CONCLUSION

The crude cellulase enzyme from *Aspergillus unguis* was purified from the spent medium by 80% saturated ammonium sulphate precipitation and by ion exchange chromatography using DEAE-sepharose fast flow and the molecular weight was determined as 50 KDa. Purified cellulase showed zero order kinetics for 5 minutes. The pH and temperature optimum for the purified cellulase were 5.0 and 70^{o}C respectively. Michaelis constant for the pure cellulase to soluble cellulose was 4.45×10^{-2}moldm^{-3} and Vmax was 28.5714mg ml^{-2}mins^{-1}at pH 5.0 and at 70^{o}C. Since the purified cellulase from *Aspergillus unguis* was active in moderately acidic pH (5.0) and showed better stability at 70^{o}C it could be considered as a potential candidate for the conversion of biomass into fuel and other industrial applications.

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