Characterization of Total Cellulase and endo- β-1, 4-glucanase and their Applications in Biofuels Production as well as Protection of Crops from Damaging by Insects

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Abstract: Efficient and low-cost cellulolytic enzymes are urgently needed to degrade recalcitrant plant biomass during the industrial production of biofuels. Nevertheless, Blepharomastix is a serious pest of okra plant as it feeds on the leaves, soft stems, fruit of okra and eventually damage okra production. Therefore, the aim of this study is to characterize the total cellulase and endo-β-1, 4-glucanase (digestive enzymes) present in the larvae of Blepharomastix as it is a popular trend to apply enzymes in biofuels production and application of enzyme inhibitors to protect crops from damaging throughout the world. The result analysis showed that the whole gut from the larvae exhibited the activities of total cellulase and endo-β-1, 4-glucanase enzymes that hydrolyzed crystalline cellulose and carboxymethyl cellulose (CMC) to glucose and the activities in insect were 0.076 µmol min⁻¹ ml⁻¹ and 0.398 µmol min⁻¹ ml⁻¹ respectively. However, the optimum temperature for the activity of total cellulase and endo-β-1, 4-glucanase in Blepharomastix were 45˚C and 50˚C respectively. The pH optima of total cellulase and endo-β-1, 4-glucanase in Blepharomastix was 9.0. The cellulase activity was inhibited by ethylenediaminetetraaceticacid (EDTA), sodium dodecyl sulphate (SDS) and urea, whereas enhanced by NaCl, KCl and MgCl₂.

Keywords: Cellulase, endo- β-1, 4-glucanase, Blepharomastix

1. INTRODUCTION

Because of the increasing demand to overcome energy shortages and achieve stable economic development, cellulose-based biofuels have recently become a major focus of industrial and academic communities worldwide [1]. During biofuel production, linear chains of cellulose, which consist of glucose residues connected by a β-1, 4 linkage, need to be degraded to glucose, which is then fermented to produce ethanol. At present, even though microbial and chemical degradation of cellulose has been widely used, the use of combined cellulolytic enzymes remains limited because of the high cost of biotechnological tools [2]. Furthermore, in industry, breakthrough technologies to overcome the barriers of developing cost-effective processes for converting biomass to fuels and chemicals have yet to be fully realized. Therefore, there is an urgent need to discover and develop more efficient cellulolytic enzymes
that reduce biofuel production cost, in addition to having applications in other industrial processes [3]. However, okra (Abelmoschus esculentus) is often known as Lady's fingers has a great nutritional and economical value throughout the world. The pod yield of lady's finger varied from 6.9 to 18.9 t ha\(^{-1}\) in relation to farmer's category in Bangladesh [4]. Besides, it helps to stabilize the blood sugar; prevents and improves constipation; filters liver to remove toxins; assures easy passage out of waste from the body; used to treat lung inflammation, sore throat and irritable bowel; used for healing ulcers and to keep joints limber; helps to neutralize acids and so on [5]. But this staple vegetable crop is extensively damaged by the larvae of insect (Blepharomastix) and losses range from 50-70% because it feeds on the leaves, soft stems and fruit of okra, also the young leaves are favored. They can destroy large sections of plants and bind parts of the leaves together with silk and faces. On occasion, the larvae have been known to damage the flowers. After the fruit starts to develop, the larvae can chew shallow holes in the surface. They often pupate in these holes. This damage causes blemishes in the fruit, which downgrades the quality [6]. The affected fruits lose their market value besides considerable reduction in yield. The pest poses a serious problem because of its high reproduction potential, rapid turnover of generations. Farmers use large quantities of chemical insecticides for effective control of the larvae. This practice of indiscriminate use of insecticides leads to build up of pesticide residues in the product, destruction of beneficial insects, pest resurgence, pesticide exposure to farm workers that cause various types of cancer and environmental pollution.

Hence, the aim of this study is to characterize the total cellulase and endo- β-1, 4-glucanase activities of Blepharomastix to gain a better understanding of the digestive physiology of the Blepharomastix. The understanding will hopefully lead to management and reduce pest-linked damage in Okra crop as well as to protect the environment from adverse effects of pesticides, deploying total cellulase specific inhibitors for Blepharomastix. In addition, insect’s total cellulase and endo- β-1, 4-glucanase could be widely used in biofuels production [2] as well as various industrial processes including food, textile and laundry, fermentation, pulp and paper [7]. Nevertheless, though there were many research works [1], [2], [8] on the total cellulase and endo- β-1, 4-glucanase had been done throughout the world, but a research work on the total cellulase and endo- β-1, 4-glucanase of Blepharomastix was the first in the world. Therefore, this research work is a noble work. Furthermore, enzymes characteristics may be altered due to environmental reasons, ecological causes, geographical locations etc. It also may be changed from species to species as different organisms have different physical, chemical and biological characteristics. Hence, the research works is logical from scientific point of views since no research works on total cellulase and endo- β-1, 4-glucanase was done before on this geographical location.

2. METHODS AND MATERIALS

2.1. Collection of larvae: The larvae of Blepharomastix that affect okra leaves and fruits (Figure-1) were collected from different okra fields of Chittagong district and kept in laboratory, maintained at normal temperature. The larvae were isolated under the feeding condition from the affected okra leaves and fruits with dissection instruments and immobilized in ice-cold bath. Then they were stored at ~20°C.

2.2. Preparation of sample: Enzyme samples from the whole gut of the larvae were prepared by the Cohen’s method [8] with slight modification. Whole gut from these individuals was removed by dissection under naked eyes in ice-cold normal saline [(0.9% NaCl w/v) MARK, India] on a petri dish which was on the ice box. The weight of the dissected whole gut were taken and found 5.10 gm. The separated whole gut from the larvae was rinsed in ice-cold normal saline and placed pre-cooled
homogenizer [IKA-WERKE GmbH and Co.Kg.d-79219 staufen] and ground in acetate buffer (0.5 ml of buffer per gm of midgut) for cellulase activity. The homogenates from preparation (whole gut) were transferred to 1.5 ml centrifuge tubes and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatants were pooled and stored at –20°C.

Figure 1. The studied larvae and adult of Blepharomastix that damage okra fruits and leaves.

2.3. Measurement of total cellulase activity: Insect gut extract of 25μl was mixed with 400μl of 5% cellulose made up in buffer. Suspension of the cellulose was ensured by vortexing the stock cellulose immediately before pipetting. Substrate blank (25μl of buffer + 400μl of cellulose) and sample blank (25μl of extract + 400μl of buffer) were also carried out with the experiment. All the contents of the reaction tube were immediately mixed by swirling and incubated at 37°C for exactly 120 minutes with moderate shaking using water bath (Grand-OLS200). After incubation all the tubes were immediately transferred into the ice bath and allowed to stand until the suspension was settled. The reaction mixtures were centrifuged at 2500 rpm for 7 minutes to clarify. 200μl of supernatant was used for estimating glucose using Arsenomolybdate chromogenic reagent of Nelson according to the Somogyi’s method. The quantity of glucose (MARK) was obtained from calibration curve of standard glucose solution (Figure-2a). Appropriate blank was run in all experiments. Absorption of samples, standards and blanks was read at 660 nm using UV spectrophotometer (Shimadzu, Japan). The experiments were repeated three times simultaneously.

2.4. Measurement endo β-1, 4-glucanase activity: Insect whole gut extract of 25μl was mixed with 225μl of 1% carboxymethyl cellulose (CMC) made up in buffer. Gradient of the CMC was removed by vortexing the stock CMC solution immediately before pipetting. Substrate blank (25 μl of buffer + 225μl of 1% CMC) and sample blank (25 μl of extract + 225μl of buffer) were also carried out with the experiment. All the contents of the reaction tube were immediately mixed by swirling and incubated at 37°C for 60 minutes using water bath. The reaction was stopped by adding 450μl of 3, 5 dinitrosalisilic acid solution (DNS) solution. The tubes were then immediately incubated at 100°C in a water bath for 5 minutes. Immediately, 230μl of 40% Rochelle salt was mixed with before cooling it at room temperature and 1.5ml of water was added. The concentrations of reducing sugar produced during the incubation were obtained from a standard curve prepared from aqueous solutions of glucose (Figure-2b). Absorption of samples, standard and blanks was read at 540 nm using UV spectrophotometer and the experiments were repeated three times simultaneously.
2.5. Time course of total cellulase activity: The time course of total cellulase activity was determined by incubating the reaction mixtures containing 25 μl of extract and 400 μl of 5% cellulose in 0.1 M sodium acetate buffer, pH 5.3 at 37°C. The reaction was carried out for 0 min, 5 min, 10 min, 20 min, 30 min, 40 min, 60 min, 90 min and 120 min. The reactions were stopped at the end of the incubation for defined time intervals. The total cellulase activity was measured as described before and the experiments were repeated three times simultaneously.

2.6. Determination of optimum temperature of total cellulose: The temperature for optimum total cellulase activity was determined by incubating the reaction mixture containing 25 μl of extract and 400 μl of 5% cellulose in 0.1 M sodium acetate buffer, pH 5.3 at 35°C, 40°C, 45°C, 50°C, 60°C and 70°C for 40 minutes. The total cellulase activity was measured as described before and the experiments were repeated three times simultaneously.

2.7. Determination of optimum temperature of endo-β-1, 4-glucanase: The optimum temperature for endo-β-1, 4-glucanase activity was determined by incubating the reaction mixture containing 25 μl of extract and 225 μl of 1% CMC in 0.1 M sodium acetate buffer, pH 5.3 at 35°C, 40°C, 45°C, 50°C, 60°C and 70°C for 60 minutes. The activity of endo-β-1, 4-glucanase was measured as described before and the experiments were repeated three times.

2.8. Determination of optimum pH of total cellulase: The optimum pH for total cellulase activity was determined by incubating the reaction mixture containing 25 μl of extract and 400 μl of 5% crystalline cellulose in buffer with different pH ranges. The dependence of activity on pH was determined by assaying the total cellulase activity with 0.1 M buffer solutions in the pH range 3.0 to 13.0. Six buffers were used: citrate buffer for pH 3.0; sodium-acetate buffer for pH 4.0 and 5.0; phosphate buffer for pH 6.0, 7.0 and 11.0; tris-HCl buffer for pH 8.0; glycine-NaOH buffer for pH 9.0 and 10.0; and KCl-NaOH buffer for pH 12.0 and 13.0. The Cellulase activity was described before but temperature was 45°C and the experiments were repeated three times.

2.9. Determination of optimum pH of endo-β-1, 4-glucanase: The pH optima for endo-β-1, 4-glucanase activity in the homogenate of the insect larvae were determined by using carboxymethyl cellulose (CMC) as substrate in buffer with different pH ranges. The dependence of activity on pH was determined by assaying the total cellulase activity with 0.1 M buffer solutions in the pH range 4.0 to 13.0 were used. Five buffers were used: Na-acetate buffer for 4.0 and 5.0; phosphate buffer for pH 6.0, 7.0 and 11.0; tris-HCl buffer for pH 8.0; glycine-NaOH buffer for pH 9.0 and KCl-NaOH buffer for pH 12.0 and 13.0. The activity of endo-β-1, 4-glucanase was assayed as described before but temperature
was 50˚C and the experiments were repeated three times simultaneously.

2.10. Determination of activators and inhibitors on total cellulase activity: To test the effect of different ions on the enzyme, whole gut of *Blepharomastix* was dissected in distilled water. Cellulase assay was performed in the presence of different concentrations of NaCl (20 and 40 mmol/L), KCl (20 and 40 mmol/L), CaCl\(_2\) (20 and 40 mmol/L), MgCl\(_2\) (20 and 40 mmol/L), ethylenediaminetetraacetic acid (EDTA) (20 and 40 mmol/L), sodium dodecylsulfate (SDS) (20 and 40 mmol/L) and urea (100 and 200 mmol/L). These compounds were added to the assay mixture, and activity was measured after 40 min incubation. A control (no compounds were added) was also measured and the experiments were repeated three times simultaneously.

3. RESULTS AND DISCUSSION

3.1 Total cellulase activity of midgut extract: Incubation of crystalline cellulose as a substrate with whole gut extract from *Blepharomastix* yielded glucose and established the presence of cellulase activity in the whole gut of *Blepharomastix*. The specific activity of the enzyme in the whole gut of *Blepharomastix* was 0.076 µmol min\(^{-1}\) ml\(^{-1}\).

3.2. Endo-β-1, 4-glucanase activity: The studies showed that endo-β-1, 4-glucocanase activity was present in the whole gut of *Blepharomastix*. The specific activity of the enzyme in the whole gut of *Blepharomastix* was 0.398 µmol min\(^{-1}\) ml\(^{-1}\).

3.3. Time course of total cellulase activity: The rate of glucose production by cellulolytic hydrolysis of crystalline cellulose is illustrated in (Figure-3). From the graph it appeared that the enzyme reaction rate on the crystalline cellulose substrate was linear until 40 minutes, after that the rate increased slightly till 90 minutes and then the rate of reaction steadily decreased until termination of the experiment at 120 minutes.

![Figure 3. Time course of total cellulase activity.](image)

3.4. Effect of temperature on the activity of total cellulase: The effect of temperature on the total cellulase activity is shown in (Figure-4a). With a reaction time of 40 minutes, the optimum temperature of the enzyme was 45˚C, and more than 60% of the maximal activity was obtained at 40˚C and 50˚C. The activity of total cellulase was dropped off at 70˚C. The activity was linear between 35˚C and 45˚C.
3.5. Effect of temperature on the activity of Endo-β-1, 4-glucanase: The effect of temperature on Endo-β-1, 4-glucanase activity is shown in (Figure 4b). With a reaction time of 60 minutes, the optimum temperature of this enzyme was 50˚C. Endo-β-1, 4-glucanase was considerably active between 40˚C and 60˚C. The activity dropped off sharply above 60˚C. A drop in endo-β-1, 4-glucanase activity occurred gradually below 50˚C with only 34.50% activity at 35˚C.

![Figure 4](image)

Figure 4. Effect of temperature on total cellulase activity (a) and endo-β-1, 4-glucanase activity (b)

3.6. Effect of pH on the activity of total cellulase: The result of hydrolysis with crystalline cellulose as a substrate indicated a short range of total cellulase activity while a sharp peak at pH 9.0. Total cellulase activity dropped off rapidly below pH 4.0 and above pH 10.0. So the activity of total cellulase was low at slightly acidic pH 5.0 and was high at alkaline pH 9.0 (Figure 5a).

![Figure 5](image)

Figure 5. Effect of pH on total cellulase activity (a) and endo β-1, 4-glucanase activity (b)

3.7. Effect of pH on the activity of endo-β-1, 4-glucanase: The activity of endo-β-1, 4-glucanase from the whole gut of Blepharomastix was affected by the pH of incubation buffer (Figure 5b). Endo-β-1, 4-glucanase was optimally active at pH 9.0. Endo-β-1, 4-glucanase activity was dropped off rapidly above pH 9.0 with only 1.45% at pH 13.0 and gradually decreased below pH 7.0 and above 9.0.

3.8. Effect of activators and inhibitors on total cellulose: The effect of various activators and inhibitor on total cellulase activity is presented in (Table 1). The table showed that NaCl, KCl and MgCl₂ increased total cellulase activity. There was no significant difference between NaCl and KCl on total cellulase activity. The highest activity was obtained with 40 mmol/L of NaCl concentration. Four compounds – EDTA, SDS, CaCl₂ and urea had an inhibitory effect on enzyme activity (Table 1). Inhibitory effects of
EDTA and SDS at a concentration of 40 mmol/L were 59.94% and 41.19%, respectively. Total cellulase activity was mildly inhibited by urea and 94.07% and 74.33% at a concentration of 100 and 200 mmol/L (we used the concentration "100 and 200" for SDS instead of "20 and 40" because low concentration could not be response to the enzyme).

**Table 1.** Relative activity of cellulase of *Blepharomastix* toward different compounds.

| Compound | Concentration (mmol/L) | Relative activity (%) |
|----------|-------------------------|----------------------|
| Control  | -                       | 100                  |
| NaCl     | 20                      | 107.89               |
|          | 40                      | 113.26               |
| KCl      | 20                      | 103.39               |
|          | 40                      | 111.57               |
| CaCl₂    | 20                      | 97.32                |
|          | 40                      | 80.68                |
| MgCl₂    | 20                      | 101.55               |
|          | 40                      | 106.06               |
| EDTA     | 20                      | 79.68                |
|          | 40                      | 59.94                |
| SDS      | 100                     | 67.70                |
|          | 200                     | 41.19                |
| Urea     | 20                      | 94.07                |
|          | 40                      | 74.33                |

After considering the results obtained in the present study, it is possible to infer that the enzymatic apparatus of the larvae of *Blepharomastix* is especially appropriate for the digestion of polysaccharides such as crystalline cellulose and carboxymethyl cellulose, which are comprised principally of glucose residues. Crystalline cellulose and carboxymethyl cellulose are the major sources of carbon for insects as well as other organisms and for this reason *Blepharomastix* may extensively attack okra crops. Moreover, total cellulase and endo-β-1, 4-glucanase activities of larval extract of *Blepharomastix*, that were collected from affected leaves and fruits, were 0.076 µmol min⁻¹ ml⁻¹ and 0.398 µmol min⁻¹ ml⁻¹, respectively which indicates okra crop may be extensively damaged by the larvae of *Blepharomastix* and highest cellulase, and endo-β-1, 4-glucanase activities also may correlate to highly feeding of insects that extensively damage crops. Cellulase activity reported for lipidopterian larvae, showed increased activity with advancing larval development [9]. In contrast, Willis [10] did not detect cellulolytic activity during development of *Dissosteira carolina*. Nevertheless, cellulolytic enzymes obtained from the larvae of *Blepharomastix* might provide invaluable foundation for efficient and low-cost cellulose degradation and ethanol biofuel production [2]. Because bringing forth a sustainable and renewable energy alternative for the future has become a great challenge for scientists, engineers and technologists due to the enormous consumption of global energy and its connection with some critical issues such as climate change [19].

*Blepharomastix* cellulase showed maximum glucose production at 45°C with crystalline cellulose substrate and endo-β-1, 4-glucanase showed maximum activity at 50°C. These results indicated that thermostable cellulase and endo-β-1, 4-glucanase could be commercially applied in various industries including food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry [11] as maximum enzyme activity at high temperatures is a very suitable characteristic for its industrial acceptability [12]. A previous study reported that the maximum activity of cellulase in *Fomitopsis pinicola* was observed at 50°C [13] and maximum activity of endo-β-1, 4-glucanase in *Myceliophthora thermophila* was investigated at 60°C [14]. On the other hand, our study revealed that we can save our crop from damaging of larvae of *Blepharomastix* by growing crops in
winter season as cellulytic enzymes of this insect is more active in high temperature. In the current study, the activities of cellulase and endo-β-1, 4-glucanase were examined across a broad range of pH, but the enzymes were only active over a limited range of pH. The highest activities of cellulase and endo-β-1, 4-glucanase of Blepharomastix were found at pH 9. These results are strongly supported by the fact that endo-β-1, 4-glucanase of Bacillus halodurans showed maximum activity at pH 9.0 [15]. Whereas, cellulase of Chaetomium thermophilum showed maximum activity at pH 5.0 [16] [17].

The results of this study indicated that these enzymes prefer alkaline pH for optimal activities and can be introduced as an industrially and economically feasible enzyme [12]. Also, it is recommended that farmers of studied area can protect their crops from damaging of larvae of Blepharomastix by spraying normal water (pH 7.0) in their okra fields instead of chemical pesticides and insecticides which is harmful for environments as well as human health since enzymes of this insect is less active at pH below 7.0. Moreover, environmental pollution due to over population, rapid industrialization and urbanization during the last decades [20] may be reduced by this spraying as pollution generated in one country with impacts in others, is now considered as a major problem in many countries of the world [21]. In this investigation, the inhibitory and activatory effect of various substances on total cellulase were studied and presented in table-1. The result analysis showed that cellulase activity was inhibited by CaCl₂, ethylene diamine tetra acetic acid (EDTA), sodium dodecyl sulphate (SDS) and urea; and their relative activities were 97.32, 79.68, 67.70 and 94.07% at low concentrations; and 80.68, 59.94, 41.19 and 74.33 % at high concentrations, respectively. These result are strongly supported by the previous study where increased concentrations of SDS and EDTA inhibited the cellulase activity in Leptinotarsa decemlineata and Lasioderma serricorne [18]. It is hoped that this study will provide a new perspective to help us in controlling of the pests using these enzyme inhibitors. On the contrary, cellulase activity was enhanced by NaCl, KCl and MgCl₂; and their relative activities were 107.89, 103.39 and 101.55 % at 20 mmol/L concentrations; and 113.26, 111.57 and 106.06 % at 40 mmol/L concentrations, respectively. Preceding study reported that increase in concentration of NaCl, KCl and MgCl₂, enhanced the activity of cellulase in Leptinotarsa decemlineata and Lasioderma serricorne [18]. Ultimately, the activators study of novel cellulytic enzymes obtained from this insect might provide invaluable foundation for efficient and low-cost cellulose degradation and ethanol biofuel production.

4. CONCLUSIONS

Blepharomastix has an efficient and complete digestive cellulase and endo-β-1, 4-glucanase system able to hydrolyze plant leaves as well as other substrates due to its highly basic enzyme system and so able to feed on different economically important crop. So, it may possible to control this insect using enzyme technology. Moreover, the biological aspects of processing of cellulosic biomass became the crux of future research involving cellulase and endo-β-1, 4-glucanase. Cellulase and endo-β-1, 4-glucanase are being commercially produced by several industries globally and are widely being used in food, animal feed, fermentation, agriculture, pulp and paper and textile applications. With modern biotechnology tools, especially in the area of enzymology, novel enzymes and new enzyme applications will become available for the various industries. Improvements in cellulase activities or imparting of desired features to enzymes by protein engineering are probably other areas where cellulase research has to advance.

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REFERENCES

[1] Sun, J.Z.; Scharf, M.E. Exploring and integrating cellulolytic systems of insects to advance biofuel technology. Insect Science, 2010, 17, 163-165.

[2] Su, L.J.; Zhang, H.F.; Yin, X.M.; Chen, M.; Wang, F.Q.; Xie, H.; Zhang, G.Z.; Song A.D. Evaluation of cellulolytic activity in insect digestive fluids. Genetics and Molecular Research, 2013, 12 (3), 2432-2441.

[3] Wyman, C.E. What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnology, 2007, 25, 153-157.

[4] Rashid, M.H.; Nur-E-Elahi, M.; Khan M.A.H. The Participation of Different Categories of Farmers for the Production and Utilization of Lady's Finger (Hibiscus esculentus L.) RFS Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh Journal of Scientific and Industrial Research, 2006, 41(1-2), 15-22.

[5] Pyro-energen, Article: http://www.pyroenergen.com/articles07/okra-health-benefits.htm Retrieved February 23, 2015.

[6] Brown, H. Entomology, Darwin Common Insect Pests of Cucurbits. 2003, Agnote. No: I59 www.nt.gov.au/dpifm ISSN 0157-8243, Serial No. 805, Agdex No. 263/622.

[7] Bhat, M.K. Cellulases and related enzymes in biotechnology. Biotechnology Advances, 2000, 18, 355-383.

[8] Cohen, A.C. Organization of digestion and preliminary characterization of salivary trypsin like enzymes in a predaceous Heteropteran, Zelus renadii. Journal of Insect physiology, 1993, 39, 823-829.

[9] Naconieczny, M.; Michaelczyk, K.; Kedziorski, A. Midgut glycosidase activities in momophagous larvae of Apollo butterfly, Parnassius Apollo ssp. frankenbergeri. Comptes Rendus Biologies, 2006, 329, 765-774.

[10] Willis, J.D.; Klingeman, D.W.E.; Oppert, C.; Oppert, B.; Jurat-Fuentes, J.L. Characterization of cellulytic activity from digestive fluids of Dissosteira carolina (Orthoptera: Acrididae). Comparative Biochemistry and Physiology, 2010, 157, 267-272.

[11] Kuhad, R.C.; Gupta, R.; Singh, A. Microbial Cellulases and Their Industrial Applications. SAGE - Hindawi Access to Research, Enzyme Research, 2011, 1-10, Article ID 280696.

[12] Sepahy, A.A.; Jabalameli, L. Effect of Culture Conditions on the Production of an Extracellular Protease by Bacillus sp. Isolated from Soil Sample of Lavizan Jungle Park. SAGE-Hindawi Access to Research, Enzyme Research, 2011, 1-7, Article ID 219628, doi:10.4061/2011/219628

[13] Shin, K.; Kim, Y.H.; Jeya, M.; Lee, J.K.; Kim, Y.S. Purification and characterization of a thermostable cellobiohydrolase from Fomitopsis pinicola. Journal of Microbiology and Biotechnology, 2010, 20(12), 1681-8.

[14] Karnaouri, A.C.; Topakas, E.; Christakopoulos, P. Cloning, expression and characterization of a thermostable GH7 endoglucanase from Myceliophthora thermophila capable of high-consistency enzymatic liquefaction. Applied Microbiology and Biotechnology, 2013, 98, 231-42.

[15] Annamalai, N.; Rajeswari, M.V.; Elayaraja, S.; Balasubramanian, T. Thermostable, haloalkaline cellulase from Bacillus halodurans CAS 1 by conversion of lignocellulosic wastes. Carbohydrate Polymers, 2013, 94(1), 409-415.

[16] Li, Y.L.; Li, H.; Li, A.N.; Li, D.C. Cloning of a gene encoding thermostable cellobiohydrolase from the thermophilic fungus Chaetomium thermophilum and its expression in Pichia pastoris. Journal of Applied Microbiology, 2009, 106 (6), 1867-1875.

[17] Linares-Pasten, J.A.; Andersson, M.; Karlsson, E.N. Thermostable Glycoside Hydrolases in Biorefinery Technologies. Current Biotechnology, 2014, 3, 26-44.

[18] Sajjadian, S.M.; Hosseinianvah, V.; Vatanparast, M. Cellulase activity in the larval digestive tract of the Colorado potato beetle, Leptinotarsa decemlineata (Coleoptera: Chrysomelidae) and the cigarette beetle, Lasioderma serricorne (Coleoptera: Anobiidae). Journal of Crop Protection, 2012, 1 (3), 201-210.

[19] Dattatray, J.L; Chandra, S.R.; Dishu, C.; Satyajit, R. Emerging Energy Applications of Two-Dimensional Layered Materials. Canadian Chemical Transactions, 2015, 3 (2), 118-157.
[20] Begum, K.; Mohiuddin, K.M.; Zakir, M.H.; Rahman, M.M.; Hasan, M.N. Heavy Metal Pollution and Major Nutrient Elements Assessment in the Soils of Bogra City in Bangladesh. Canadian Chemical Transactions, 2014, 2 (3), 316-326.

[21] Saadat, A.M.H.; Md. Mahfuzur Rahman, M.M.; Hasan, S.M.K; Jahangir Alam, A.T.M.J. Travelling and Source Point Identification of Some Transboundary Air Pollutants by Trajectory Analysis in Sathkhira, Bangladesh. Canadian Chemical Transactions, 2013, 1 (1), 56-65.

The authors declare no conflict of interest

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