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Enhanced production of cellulose degrading CMCase by newly isolated strain of *Aspergillus versicolor*

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ABSTRACT

Production of CMCase from newly isolated strain of *Aspergillus versicolor* was studied by optimizing different physico-chemical parameters. Cellulolytic potential of the isolate was confirmed by the simple agar plate method and then subjected to variation of a single parameter at a time in CMC containing medium. Maximum enzyme production was obtained at 30 °C in 120 h when medium was supplemented with 0.5 gm% carboxymethyl cellulose, 0.2 gm% Tween 80, 0.075 gm% peptone, 0.050 gm% $CaCl_2$, 1.5 gm% $NaNO_3$ and 0.2 gm% KH_2PO_4 at pH 4.0. Current study holds great potential as the organism reported here could easily be used for the production of cellulase using renewable biomass.

1. Introduction

Keeping in view the importance of cellulase enzymes, exploitation of indigenous resources for the isolation of better strains of cellulase producing organisms is the need of time for industrial purpose (Mandels & Reese, 1999; Bhat, 2000). Industrial usage of cellulases and hemicellulases first started from animal feed which than followed its way into various food applications (Voragen, Heutink, & Pilnik, 1980). Fungi recycle degradable organic waste into important plant nutrients and many types of them are capable of converting even cellulosic wastes (Linko, 1977; Kim, Yoo, Oh, & Kim, 2003). The biological degradation of cellulose has great importance in the activity of living system. Various carbohydrases such as amylases and cellulases are commonly used in textile, detergent and baking industries represent the second largest group of enzymes (Godfrey & West, 1996). Many cellulolytic products which are not useful for human consumption are converted into useful products by the help of microorganism. Cellulolytic fungi are most suited option for cellulase production because of easy handling and economically feasible process as compared to other sources (Siddiqui, Saqib, Rashid, & Rajoka, 2000; Lee & Koo, 2001).

Fungi can be easily grown in a medium comprising inorganic nutrients and a protein source for healthy growth and spore formation (Mandels & Reese, 1999; Buzzini & Martini, 2002). Temperature, pH and incubation period of different fungal strains plays critical role in production and stability of different enzymes and other metabolites (Juhász, Szengyel, Szijártó, & Réczey, 2004). Fungi can be cultured upon required different kinds of fermentative media; both submerged and solid state for the production of different enzymes (Gautam et al., 2011). Submerged medium usually selected for analytical purpose where rapid growth in liquid medium takes place due to equal distribution of nutrients for maximum fungal growth (Bisaria & Ghose, 1981). On industrial scale solid state medium is preferred where the bulk quantities of enzymes are required with low analytical grade quality (Lynd, Weimer, van Zyl, & Pretorius, 2002). Major portion of lignocellulosic wastes are produced from agriculture and forest and also from industrial sources such as textile paper pulp, breweries, and timber industries and these waste causing a major role in environmental pollution. However if these lignocellulosic are biologically degraded valuable products such as biofuels, animal feeds and human nutrient materials can be produced on large scale (Acharya, Acharya, & Modi, 2008; Jabbar & Ilahi, 1981).

2. Material and methods

2.1. Sampling

Soil samples were collected from paper reprocessing areas and sugarcane field. All samples collected in sterilized polyethylene bags, labeled and stored at 4 °C for further analytical work.

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2.2. Isolation and purification of fungi

Sterilized potato dextrose agar (PDA) plates were used for isolation purpose and all isolates after purification were kept in PDA slants at 4 °C. Czapek Dox media plates were used for purification and identification of isolates. Sub culturing of pure isolates performed periodically after every 20 days. After series of experiments, *Aspergillus versicolor* was selected for submerged culture optimization (Wu, Ding, & Zhang, 2006)

2.3. Screening of isolated fungi

Aspergillus versicolor was grown in 1% CMC containing medium for screening purpose using plating method (Hankin & Anagnostakis, 1977). *Aspergillus versicolor* were streaked on medium plates and incubated at 30 °C for 72 h than plates were flooded with 0.5% congo red for 15 min and washed with 1 M NaCl solution twice to remove excess dye. The appearance of clear zone around colonies showed positive result for cellulase production.

2.4. Enzyme assay

Carboxymethyl cellulase (CMCase) was estimated using 1.0 ml substrate (0.5% CMC in 50 mM citrate phosphate buffer pH 5.00) was added in 0.5 ml of enzyme extract and incubated at 30 °C for 30 min (Mandels & Andreotti, 1978). Reaction was stopped by adding 1.0 ml DNS and reducing sugars were estimated (Miller, 1959).

One unit (IU) of CMCase activity was defined as the amount of enzyme required to release 1.0 μmol of glucose per minute.

2.5. Effect of Incubation time on enzyme production

Innocula (50 ml) of 48 h was transferred in different flasks of 450 ml media and incubated at 30 °C for different time intervals ranging from 24–168 h (7 days) and after every 24 h CMCase activity was performed. (Millati, Niklasson, & Taherzadeh, 2002).

2.6. Effect of CMC concentration on enzyme production

Maximum carboxymethyl cellulase (CMCase) production with different concentration of CMC ranging from 0.25–1.5% was optimized in separate 250 ml Erlenmeyer flasks keeping the other constituent of medium constant.

2.7. Effect of peptone on enzyme production

Different concentrations of peptone ranging from 0.025–0.15% (0.025%, 0.05%, 0.075%, 0.1%, 0.125%, and 0.15%) were added in separate flasks for maximum production of CMCase before autoclaving. These media were incubated for five days at 30 °C.

2.8. Effect of different salts on enzyme production

Different concentrations of $CaCl_2$ (0.025, 0.050, 0.075 and 0.1%) were added in fermentation media separately before sterilization. Sodium nitrate ($NaNO_3$) in different concentrations (0.25, 0.5, 1.0, 1.5 and 2.0%) was also added in the fermentation broth. Similarly, effect of potassium dihydrogen phosphate (KH_2PO_4) on CMCase production was investigated by incorporating different concentrations (0.1, 0.2, 0.3, and 0.4%) in fermentation media to select best concentration for maximum production of CMCase.

2.9. Effect of temperature on enzyme production

Maximum production of carboxymethyl cellulase (CMCase) at different temperature was carried out ranging from 20 °C to 60 °C keeping the medium composition constant.

2.10. Effect of pH on enzyme production

Maximum production of carboxymethyl cellulase (CMCase) at different pH was achieved using same media composition having different pH ranging from 4.0 to 7.0 and pH was adjusted using HCl/NaOH before sterilization. After sterilization equal amount of spore suspension was inoculated for five days at 30 °C (Farinas et al., 2010).

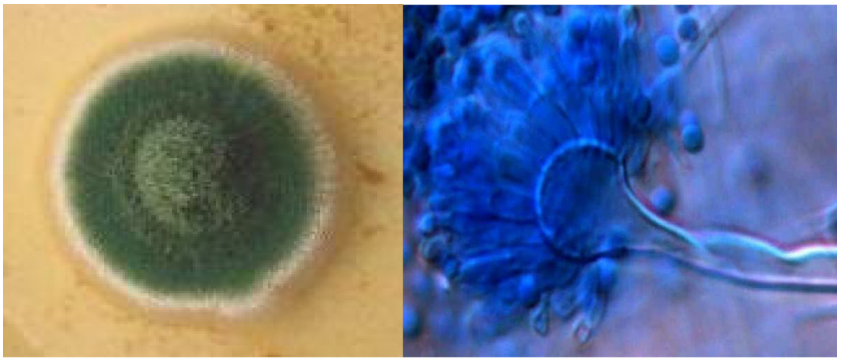
3. Results and discussion

3.1. SEM of Aspergillus versicolor

Different cultures of fungi were isolated from different sources and streaked on medium plates and incubated at 30 °C for 72 h. After incubation enzyme activity of different fungi were performed and it was observed that *Aspergillus versicolor* showed maximum CMCase activity. SEM of colonies and spores of this isolated *Aspergillus versicolor* were performed (Fig. 1).

3.2. Time course for CMCase production

The *Aspergillus versicolor* strain was incubated on CMC containing medium for maximum CMCase production at different time



A B

Fig. 1. Microscopic analysis of *Aspergillus versicolor* (A) colony (B) sporangium.

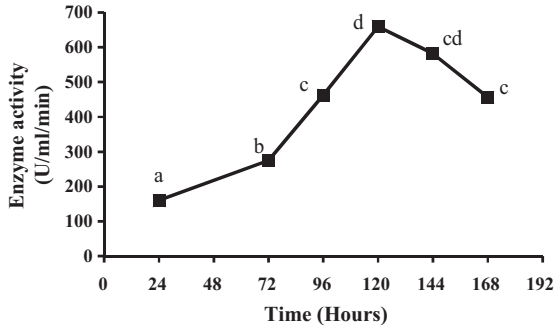


Fig. 2. Effect of different time interval on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

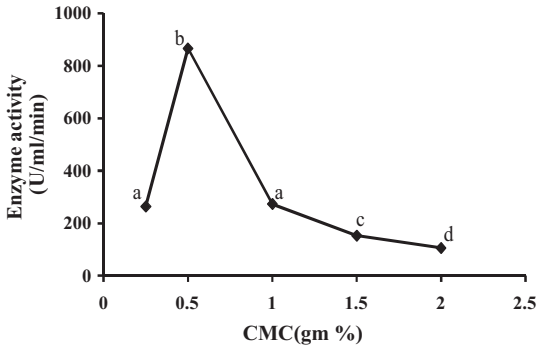


Fig. 3. Effect of different carboxymethyl cellulose concentrations on CMCase production. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

periods. It was found that the maximum enzyme production was found after 120 h and as the time increased up to 168 h, a decline in enzyme production was noted (Fig. 2). It was also observed that the increased in cell growth also increased the enzyme production. This increased in cell growth started after 24 h and reached to maximum up to 120 h directly related to enzyme production. Decreased in enzyme production after 120 h is might be due to the metabolites repressions in the fermentation medium. It was reported earlier that the maximum CMCase production was found in 120 h from *Aspergillus japonicus* URM5620 (Herculano et al., 2011) and similarly cellulase production was also reported in 72 h from fungus isolated from a rain forest (Vega et al., 2012). Different bacterial strains are also capable to produce CMCase and it was reported that the *Bacillus pumilus* EB3 produced CMCase in 24 h (Ariffin, Abdullah, Umi Kalsom, Shirai, & Hassan, 2006).

3.3. Effect of substrate concentration on CMCase production

Carboxymethyl cellulose specific medium is known to induce the production of CMCase enzyme as reported by many scientists (Acharya et al., 2008; Ahmed, Bashir, Saleem, Saddia, & Jamil, 2009; Ramanathan, Banupriya, & Abirami, 2010). In case of native *Aspergillus versicolor*, maximum CMCase production was achieved when carboxymethyl cellulose (0.5%) was used as sole carbon source in fermentation medium. It was also found that as the concentration of CMC increased, CMCase production decreased drastically (Fig. 3). This decreased in CMCase production was in fact due to the substrate feedback inhibition which activated due to the high concentration of CMC. It was also reported that higher substrate concentration resulted in poor fungal growth and low CMCase production (Liu & Yang, 2007) which might be due to the high viscosity of growing medium resulting in improper nutrient

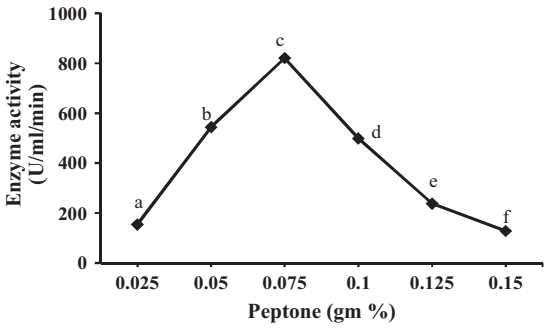


Fig. 4. Effect of peptone concentration on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

circulation; hence low fungal growth and enzyme production It was reported that the carbon sources induce production of CMCase but amount of enzyme produced is variable. This is because of the influence of substrate (carbon source) on the growth of organisms (Mandels & Reese, 1999; Zhu et al., 1982; Lakshmikant & Mathur, 1990).

3.4. Effect of peptone concentration on CMCase production

Nitrogen source is one of the important factors influencing the production of different commercial enzymes (Singh, Sharma, & Soni, 2011). Different CMCase production from *Aspergillus versicolor* was found at different concentrations of peptone in fermentation medium. Various concentrations of peptone were incorporated in separate fermentation medium and it was found that maximum production of CMCase was achieved when 0.075% peptone was incorporated in the fermentation medium. On other hand, media having more peptone concentration beyond 0.075% showed more than 60% decreased in CMCase production and continue to decrease as the peptone concentration of medium increased (Fig. 4). It was also reported that beside carbon and nitrogen sources some other factors such as temperature, pH, different salt concentrations are also affected the maximum production of enzymes (Immanuel, Dhanusha, Prema, & Palavesam, 2006).

3.5. Effect of $NaNO_3$ concentration on CMCase production

Different inorganic sources and their specific quantities in the fermentation medium also play an important role for the production of CMCase from *Aspergillus versicolor* (Wang, Li, Chen, & Liu, 2009). Different concentrations of $NaNO_3$ were added in separate fermentation medium and it was found that maximum enzyme production was achieved at 1.5%. It was reported that addition of 2.0% $NaNO_3$ in the fermentation medium increased the production of cellulase from *Aspergillus versicolor* MKU3 (Jeya, Thiagarajan, & Gunasekaran, 2005). It was found that as the concentration of $NaNO_3$ increased up to 1.5%, maximum enzyme production was achieved and further increased beyond this concentration, slight decreased enzyme production (Fig. 5). It was reported that high concentration of different salts increases the pH of the medium resulting in the sharp decline of enzyme production (Lin, Kan, Yan, & Wang, 2012). It was also reported that high concentration of nitrogenous compounds alters the hydrophobicity of cell wall which ultimately decreases the production of CMCase (Muthuvelayudham & Viruthagiri, 2006).

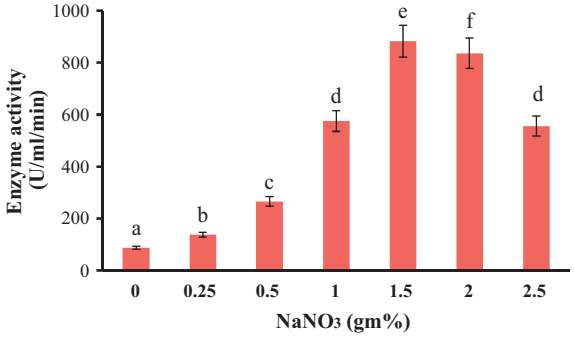


Fig. 5. Effect of $NaNO_3$ on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

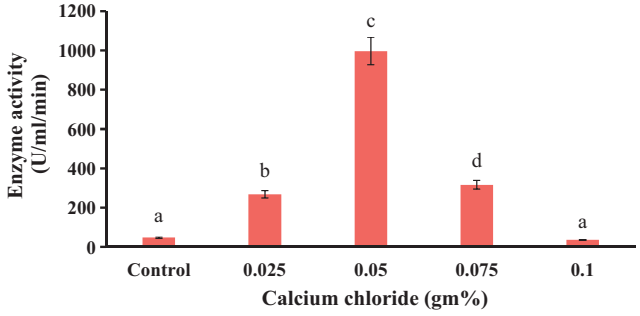


Fig. 6. Effect of $CaCl_2$ on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

3.6. Effect of $CaCl_2$ concentration on CMCase production

During current study, it was found that as the concentration of $CaCl_2$ increased in fermentation medium, increased in enzyme production was observed and maximum enzyme production was achieved at 0.05% (Fig. 6). It has been reported that addition of calcium chloride in the fermentation medium increases and stabilizes the activity of many enzymes (Mason & Jeffries, 1993; Qader, Aman, Bano, Syed, & Azhar, 2008). Bajpai (1999) also reported the use of 0.02% $CaCl_2$ in the fermentation medium for maximum production of cellulase. This positive effect of calcium chloride on enzyme production and stabilization might be due to nature of $CaCl_2$ which in the liquid state splits to provide bioavailable calcium and chloride ions which resists against pH change during fermentation process and keep the enzyme intact and stable (Asghar, Azhar, Rafiq, Sheikh, & Asad, 2002).

3.7. Effect of temperature on CMCase production

In current study *Aspergillus versicolor* was cultured at various temperatures by keeping other variables constant. It was found that at 20 °C comparatively low amount of CMCase (502 U/ml/min) was produced while at 30 °C, maximum CMCase production (897 U/ml/min) was achieved. As the temperature further increased up to 60 °C, continuous decreased in CMCase production was observed (Fig. 7). It was reported that maximum cellulase production from *Aspergillus niger* was achieved at 20 °C and as the temperature increased upto 40 °C, decreased in 48% of cellulase production was observed (Sakthi, Saranraj, & Rajasekar, 2011). It might be due to the non-availability of required oxygen in the fermentation medium and at high temperatures the solubility of oxygen decreases, resulting in limited quantity of dissolved oxygen in the medium hence anaerobic condition prevail thus making aerobic strain impossible to grow (Stewart & Parry, 1981; Rao, Mithal, Thakkur, & Sastry, 1983).

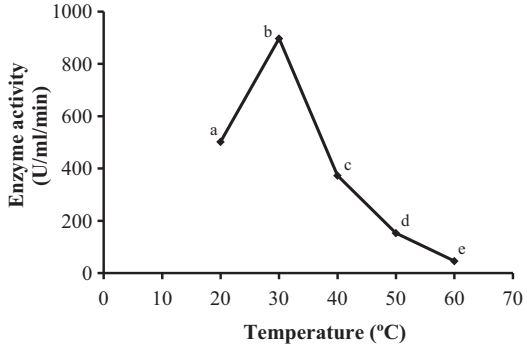


Fig. 7. Effect of temperatures on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

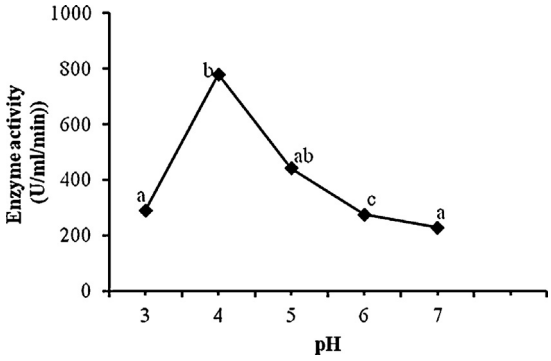


Fig. 8. Effect of pH on CMCase production. Symbols (mean ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

3.8. Effect of pH on CMCase production

Effect of pH on CMCase production was performed at different pH ranging from 3.0 to 7.0 and it was found that maximum activity was obtained at pH 4.0 (acidic media) with activity of 780 U/ml/min. It was found that production of CMCase increased up to 269% when pH was raised from 3.0 to 4.0 but when pH was raised from 4.0 to 5.0 suddenly decreased in CMCase production (57%) was noted which continue to declined as the pH increased from 5.0 to 7.0 (Fig. 8). It was previously reported that maximum CMCase activity was obtained at pH 4.0 (Prasetyo, Sumita, Okuda, & Park, 2010) and it was also reported that in case of cellulase production by *Bacillus pumilus* EWBCM1 (isolated from earthworm mid-gut), maximum cellulase production was recorded at pH 6.0 and minimum cellulase production was recorded at pH 3.0 (Shankar & Isaiarasu, 2011). It was also reported that cellulases from *Bacillus thuringiensis* showed maximum activity under acidic conditions with optimum pH at 4.0 and presented relatively wider pH-adaptability, showing more than 20% of maximum activity from pH 3.0 to 7.0 (Lin et al., 2012).

4. Conclusion

At present, cellulases and related enzymes are used in food, brewery and wine, animal feed, textile and laundry, pulp and paper industries, as well as in agriculture and for research purposes. In current study, *Aspergillus versicolor* was isolated and optimized for maximum production of CMCase. Newly isolated strain showed it maximum enzyme production at 30 °C when medium pH was kept at 4.00 before sterilization.

References

Acharya, P. B., Acharya, D. K., & Modi, H. A. (2008). Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate. *African Journal of Biotechnology*, *7*, 4147–4152.

Ahmed, S., Bashir, A., Saleem, H., Saddia, M., & Jamil, A. (2009). Production and purification of cellulose-degrading enzymes from a filamentous fungus *Trichoderma harzianum*. *Pakistan Journal of Botany*, *41*, 1411–1419.

Ariffin, H., Abdullah, N., Umi Kalsom, M. S., Shirai, Y., & Hassan, M. A. (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology*, *3*, 47–53.

Asghar, M., Azhar, U., Rafiq, S., Sheikh, M. A., & Asad, M. J. (2002). Production of α-amylase by *Arachniotus* sp. using waste bread medium. *International Journal of Agriculture and Biology*, *4*, 26–28.

Bajpai, P. (1999). Application of enzymes in the pulp and paper industry. *Biotechnology Progress*, *15*, 147–157.

Bhat, M. K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, *18*, 355–383.

Bisaria, V. S., & Ghose, T. K. (1981). Biodegradation of cellulosic materials: Substrates, microorganisms, enzymes and products. *Enzyme and Microbial Technology*, *3*, 90–104.

Buzzini, P., & Martini, A. (2002). Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. *Journal of Applied Microbiology*, *93*, 1020–1025.

Farinas, C. S., Loyo, M. M., Junior, A. B., Tardioli, P. W., Neto, V. B., & Couri, S. (2010). Finding stable cellulase and xylanase: Evaluation of the synergistic effect of pH and temperature. *New Biotechnology*, *27*, 810–815.

Gautam, S. P., Bundela, P. S., Pandey, A. K., Khan, J., Awasthi, M. K., & Sarsaiya, S. (2011). Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. *Biotechnology Research International*, *2011*, 810425.

Godfrey, T., & West, S. I. (1996). Introduction to industrial enzymology. In T. Godfrey, & S. West (Eds.), *Industrial Enzymology* (2nd ed., pp. 1–8). London: Macmillan Press.

Hankin, L., & Anagnostakis, S. L. (1977). Solid media containing carboxymethylcellulose to detect CX cellulase activity of micro-organisms. *Microbiology*, *98*, 109–115.

Herculano, P. N., Porto, T. S., Moreira, K. A., Pinto, G. A. S., Souza-Motta, C. M., & Porto, A. L. F. (2011). Cellulase production by *Aspergillus japonicus* URM5620 using waste from castor bean (*Ricinus communis* L.) under solid-state fermentation. *Applied Biochemistry and Biotechnology*, *165*, 1057–1067.

Immanuel, G., Dhanusha, R., Prema, P., & Palavesam, A. (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environment Science and Technology*, *3*, 25–34.

Jabbar, A., & Ilahi, A. (1981). Evaluation of solid substrates for the biosynthesis of cellulase by *Trichoderma viride*. *Agricultural and Biological Chemistry*, *45*, 1719–1720.

Jeya, M., Thiagarajan, S., & Gunasekaran, P. (2005). Improvement of xylanase production in solid-state fermentation by alkali tolerant *Aspergillus versicolor* MKU3. *Letters in Applied Microbiology*, *41*, 175–178.

Juhász, T., Szengyel, Z., Szijártó, N., & Réczey, K. (2004). Effect of pH on cellulase production of *Trichoderma reesei* RUT C30. *Applied Biochemistry and Biotechnology.*, *113*, 201–211.

Kim, K. C., Yoo, S. S., Oh, Y. A., & Kim, S. J. (2003). Isolation and characteristics of *Trichoderma harzianum* FJ1 producing cellulases and xylanase. *Journal of Microbiology and Biotechnology*, *13*, 1–8.

Lakshmikant, K., & Mathur, S. N. (1990). Cellulolytic activities of *Chaetomium globosum* on different cellulosic substrates. *World Journal of Microbiology and Biotechnology.*, *11*, 23–26.

Lee, S. M., & Koo, Y. M. (2001). Pilot-scale production of cellulase using *Trichoderma reesei* Rut C-30 in fed-batch mode. *Journal of Microbiology and Biotechnology*, *11*, 229–233.

Lin, L., Kan, X., Yan, H., & Wang, D. (2012). Characterization of extracellular cellulose-degrading enzymes from *Bacillus thuringiensis* strains. *Electronic Journal of Biotechnology*, *15*(3).

Linko, M. (1977). An evolution of enzymatic hydrolysis of cellulosic materials. *Advances in Biochemical Engineering*, *5*, 25–41.

Liu, J., & Yang, Y. (2007). Cellulase production by *Trichoderma koningii* AS3. 4262 in solid-state fermentation using lignocellulosic waste from vinegar industry. *Food Technology and Biotechnology*, *45*, 420–425.

Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, *66*, 506–577.

Mandels, M., & Andreotti, R. E. (1978). Problems and challenges in the cellulose to cellulase fermentation. *Process Biochemistry*, *13*, 6–13.

Mandels, M., & Reese, E. T. (1999). Fungal cellulases and the microbial decomposition of cellulosic fabric. *Journal of Industrial Microbiology and Biotechnology*, *22*, 225–240.

Mason, S. J., & Jeffries, P. (1993). The effect of calcium on activation and induction of the pectolytic enzymes produced by *Colletotrichum gloeosporioides*. *Acta Horticulturae*, *341*, 377–387.

Millati, R., Niklasson, C., & Taherzadeh, M. J. (2002). Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by *Saccharomyces cerevisiae*. *Process Biochemistry*, *38*, 515–522.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*, 426–428.

Muthuvelayudham, R., & Viruthagiri, T. (2006). Fermentative production and kinetics of cellulase protein on *Trichoderma reesei* using sugarcane bagasse and rice straw. *African Journal of Biotechnology*, *5*, 1873–1881.

Prasetyo, J., Sumita, S., Okuda, N., & Park, E. Y. (2010). Response of cellulase activity in pH controlled cultures of the filamentous fungus *Acremonium cellulolyticus*. *Applied Biochemistry and Biotechnology*, *162*, 52–61.

Qader, S. A. U., Aman, A., Bano, S., Syed, N., & Azhar, A. (2008). Role of calcium ions and temperature on dextransucrase production. *Indian Journal of Biotechnology*, *7*, 404–406.

Ramanathan, G., Banupriya, S., & Abirami, D. (2010). Production and optimization of cellulase from *Fusarium oxysporum* by submerged fermentation. *Journal of scientific and industrial research*, *69*, 454–459.

Rao, M. N. A., Mithal, B. M., Thakkur, R. N., & Sastry, K. S. M. (1983). Solid state fermentation for cellulase production by *Pestalotiopsis versicolor*. *Biotechnology and Bioengineering.*, *25*, 869–872.

Sakthi, S. S., Saranraj, P., & Rajasekar, M. (2011). Optimization for cellulase production by *Aspergillus niger* using paddy straw as substrate. *International Journal of Advanced Scientific and Technical Research*, *1*, 69–85.

Shankar, T., & Isaiarasu, L. (2011). Cellulase production by *Bacillus pumilus* EWBCM1 under varying cultural conditions. *Middle-East Journal of Scientific Research*, *8*, 40–45.

Siddiqui, K. S., Saqib, A. A. N., Rashid, M. H., & Rajoka, M. I. (2000). Carboxyl group modification significantly altered the kinetic properties of purified carboxymethylcellulase from *Aspergillus niger*. *Enzyme and Microbial Technology*, *27*, 467–474.

Singh, S., Sharma, V., & Soni, M. L. (2011). Biotechnological applications of industrially important amylase enzyme. *International Journal of Pharma and Bio Sciences*, *2*, 486–496.

Stewart, J. C., & Parry, J. B. (1981). Factors influencing the production of cellulase by *Aspergillus fumigatus* (Fresenius). *Journal of General Microbiology*, *125*, 33–39.

Vega, K., Villena, G. K., Sarmiento, V. H., Ludena, Y., Vera, N., & Gutiérrez-Correa, M. (2012). Production of alkaline cellulase by fungi isolated from an undisturbed rain forest of Peru. Biotechnology Research International, 2012, Article ID 934325.

Voragen, A. G. J., Heutink, R., & Pilnik, W. (1980). Solubilization of apple cell walls with polysaccharide degrading enzymes. *Journal of Applied Biochemistry*, *2*, 452–468.

Wang, F., Li, F., Chen, G., & Liu, W. (2009). Isolation and characterization of novel cellulase genes from uncultured microorganisms in different environmental niches. *Microbiological Research*, *164*, 650–657.

Wu, J., Ding, Z. Y., & Zhang, K. C. (2006). Improvement of exopolysaccharide production by macro-fungus *Auricularia auricula* in submerged culture. *Enzyme and Microbial Technology*, *39*, 743–749.

Zhu, Y. S., Wu, Y. Q., Chen, W., Tan, C., Gao, J. H., Fei, J. X., & Shih, C. N. (1982). Induction and regulation of cellulase synthesis in *Trichoderma* mutant EA_3-867 and N_2-78. *Enzyme and Microbial Technology*, *4*, 3–12.